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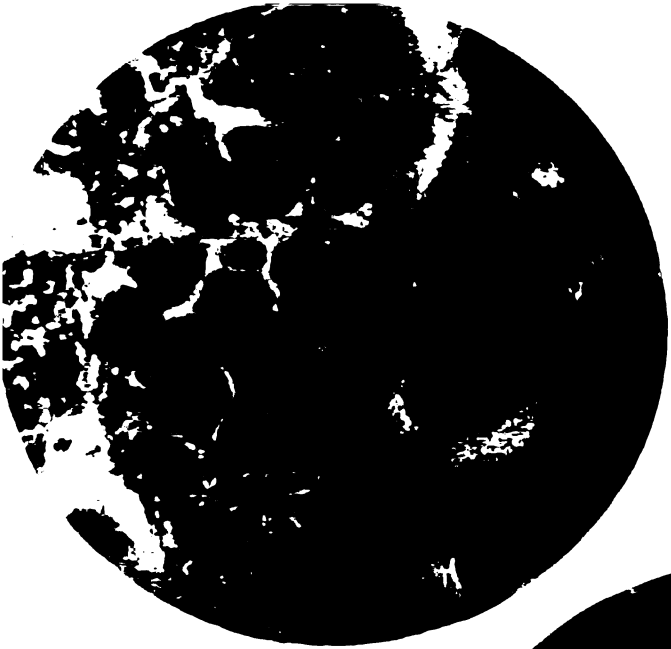

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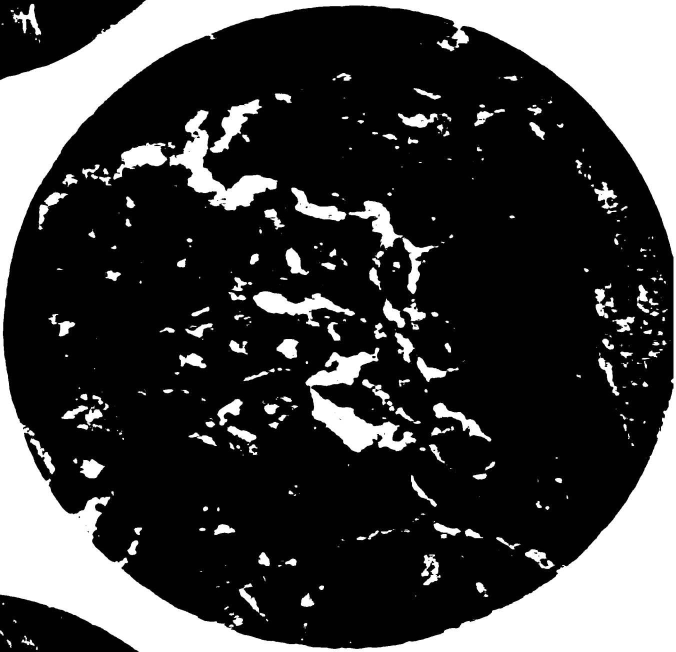

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PRODUCTS OF PROLONGED BACTERIAL ACTION ON PROTEINS.[1]

A. E. AUSTIN, M.D.

(*From the Laboratory of Physiological Chemistry, Tufts College.*)

Among the first to isolate and classify the aromatic bodies produced from albuminous bodies by bacterial action was Salkowski, whose scheme for their separation is a masterpiece, and has never been surpassed. His labors were devoted to the products of general bacterial infection from the air, or the solutions were often infected with small portions of feces in order to procure an abundant growth of these germs. In his description he mentions peptone and albumoses, without going into a more definite description of their nature or kinds. Later Nencki examined carefully the products of the action of pure growths of bacteria, both aerobic and an anaerobic, but still devoted his attention to the aromatic bodies, a very wide field, of course, for investigation. Bauman also devoted a great amount of his time to the investigation of the same subject, more particularly, however, to their production in the intestines, and first demonstrated the pairing of indol, phenol, etc., with sulphuric acid. My own interest was aroused not so much by these aromatic products as in the changes the albumin molecule undergoes in reaching this stage of disintegration, while it also seemed desirable to determine whether the process could be carried beyond the stage of the amido acids and the synchronous production of aromatics by bacterial action, as it can by splitting with strong alkalis and acids, aided by heat. The first point could be determined by

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[1] Received for publication, Nov. 13, 1902.

observing whether all the albumoses and peptones were present which are ordinarily to be found in a tryptic digestion, since the enzymic action of the bacteria resembles that of trypsin rather than that of pepsin. Fermi and Pampersi (Sulla pretesa peptoniziazione dell'albumina, Il Policlinico, 1897, No. 8) declare that the micro-oganisms containing a proteolytic enzyme as well as those that do not are not able to convert albumin into peptone, contrary to the generally accepted opinion. Further, that the proteolytic enzyme of bacteria is able to dissolve albumins, but not to peptonize them, and that, in the putrefaction of albumins, no peptone is formed.

The second question could be answered by determining whether the hexone bases, histidin, arginin, and lysin were found, substances which are produced only by the most energetic splitting of albumins by means of cooking with strong acids. These bodies, which were first found by Kossel in the nucleo-proteids, have since been found in almost all albumins, both of vegetable and animal origin. With the thought in mind that the nucleo-proteids must be used, my first efforts were made with pigs' brains, which were first run through a sausage machine at the slaughter house, and finely macerated.

Pigs' Brains.—Fifty pigs' brains, treated as above, were suspended in fifty liters of water and cooked in an autoclave under forty-five pounds pressure for six hours, removed, filtered hot, and the residue washed repeatedly with a weak alkaline solution (NaOH). Practically, this should have freed the brains from the greater part of the fats (washed out as soap), from the carbohydrate (converted to soluble sugars), and from the albumoses (atmid), which are soluble in weak alkalis, leaving chiefly the nucleins which are acted on with difficulty by superheated steam. That the filtrate and wash water contained a large amount of atmid albumose could be demonstrated by the addition of nitric acid, which caused a copious precipitate that could be redissolved by heat. The residue was suspended in a sodium carbonate solution of three-tenths per cent strength, of which the total volume was about ten liters, and left at the temperature of the room for three months, being shaken occasionally. The solid material in suspension grew less and less, more and more became liquefied, until there was only a slight layer of solid material at the bottom of the vessel, and the fluid assumed three distinct layers, which rapidly reassumed this peculiar formation after being shaken. These were carefully siphoned off, throwing away the portions on the dividing line between two layers, which was probably a mixture of both, and severally poured into separate vessels, and hereafter reference will be made to layers one, two, and three, counting from above downward.

Layer One. — This was first filtered, a wearisome undertaking, once through linen, and then with an exhaust pump through best S & S filter paper, which required to be frequently renewed on account of the fact that the albuminous particles filled the pores of the filter paper and delayed the filtration. The residue was thoroughly washed with water, and this solution was manipulated according to the scheme of Pick for the isolation of the different products of gastric digestion. The solution was somewhat cloudy, probably from the bodies of the bacteria which had passed the filter paper. The residue upon the filter paper was extracted with a ten per cent sodium chloride solution, and the residue still left dissolved by five per cent sodium hydrate solution, which left practically nothing upon the filter. These will be referred to as the watery, the salt, and the sodium hydrate solutions. The first was precipitated by twice its volume of alcohol and filtered shortly after. The precipitate was almost, but not quite, soluble in water, while the insoluble portion was soluble in five per cent sodium hydrate, and gave the biuret reaction. This latter portion was unquestionably due to the detritus from the bodies of the bacteria present. A further peculiarity was noted, that a first formed precipitate by alcohol would be redissolved by a further addition of alcohol, if this took place slowly, only to be followed by a heavier precipitation, an evidence that some of the products are more soluble in a mixture of alcohol and water than in water alone, a peculiarity which Pick has declared belongs to the group of proto-albumoses. That portion which could be redissolved in water was repeatedly redissolved in water and re-precipitated by alcohol until it was pure white. It then dissolved very rapidly in water, especially when warmed, and this solution was used for the tests given in the table appended.

The alcoholic watery solution was freed from alcohol, the whole evaporated to dryness, redissolved in water, which occurred rapidly in almost any proportion, and precipitated by an equal amount of saturated ammonium sulphate solution, *i.e.*, half saturated solution. This precipitate formed was fairly abundant, and after being well washed upon the filter with half saturated ammonium sulphate solution was dissolved in water and the ammonium sulphate removed by heat and the addition of barium hydrate. The barium sulphate formed, removed, and the excess of barium removed by carbon dioxide, the solution of the albumose was concentrated and used for the appended tests. The filtrate from the half saturation was fully saturated with ammonium sulphate in substance, producing an extensive precipitate, which was filtered off, redissolved in water, and the process repeated three times, the ammonium sulphate removed as above, and the solution somewhat concentrated and used for tests. A small portion was treated with one-tenth its volume of concentrated sulphuric acid, and again fully saturated with the same salt, but no further precipitate was formed. The greater part of the filtrate from the full saturation was freed from ammonium sulphate and also used for the tests after concentration.

Layer Two. — This was extracted in exactly the same way as the former, but a very large portion of the first alcoholic precipitate could not be redissolved in water, and was readily seen to be crystalline, forming long needles. These were purified by solution in weak ammonia and recrystallization from the fluid evaporated at the temperature of the room. They then gave the reaction with Millon's reagent and were further identified by furnishing the amount of nitrogen required by the formula of tyrosin. This peculiar grouping of different layers, in which tyrosin seeks the middle layer, has also been noted by R. L. Emerson (Beitraege zur chemischen Physiologie und Pathologie, Vol. I., p. 501) in pancreatic digestion; no leucin was found.

Layer Three.—The third layer was separated in an exactly similar way, and after these bodies had been purified the ordinary tests for albumoses were applied to them, and the results are appended in the following tables:

LAYER I.

Dys. alb., Hetero alb., Proto alb., 2nd alb.

Reagent.	Sol. NaOH.	Sol. 10% NaCl.	Pptd. by Alcohol.	Pptd. by ½ Sat'n $(NH_4)_2SO_4$	Pptd. full Sat'n $(NH_2)_2SO_4$	Non-pptd. by $(NH_4)_2SO_4$
HNO_3	+	—	—	—	—	—
Acetic and NaCl Sol.	+	—	—	—	—	—
Molisch	+	—	—	—	—	—
Biuret	+	+	+	+	+	+
Acetic and Pot. Ferro	+	—	+	+	+	—
Sat. Sol. $(NH_4)_2SO_4$	+	—	+	+	—	—
Sat. Sol. $ZnSO_4$ and H_2SO_4	+	—	—	—	—	—
Acetic	+	—	—	—	—	—

LAYER II.

Reagent.	Sol. NaOH.	Sol. 10% NaCl.	Pptd. by Alcohol.	Pptd. by ½ Sat'n $(NH_4)_2SO_4$	Pptd. full Sat'n $(NH_2)_2SO_4$	Non-pptd. by $(NH_4)_2SO_4$
HNO_3	+	—	—	+	—	—
Acetic and NaCl Sol.	+	—	—	—	—	—
Millon	+	—	+	—	—	—
Molisch	+	—	—	—	—	—
Biuret	+	+	+	+	+	—
Acetic and Pot. Ferro	+	—	+	+	+	—
Sat. Sol. $(NH_4)_2SO_4$	+	+	+	+	—	—
Sat. Sol. $ZnSO_4$ and H_2SO_4	+	+	—	+	—	—
Acetic	+	—	—	—	—	—

Layer III.

HNO_3	+	+	−	+	+	−
Acetic and $NaCl$	+	+	−	+	−	−
Acetic and Pot. Ferro.	+	+	+	+	+	
Millon	+	+	−	−	+	−
Biuret	+	+	+	+	−	
$(NH_4)_2SO_4$	+	+	−	−	−	
$ZnSO_4$ and H_2SO_4	+	+	−	−	−	−
Acetic	+	+	−	−	−	−

It was the invariable rule that all precipitates or cloudiness — and the latter is also designated by the plus sign — were soluble in heat only to return upon cooling. Before regarding the nitric acid test as negative, if it did not produce a cloudiness, half the volume of saturated salt solution was added, but in no case did it produce a precipitate where none had previously been; where reference is made to zinc sulphate and ammonium sulphate it is meant that an equal amount of the saturated solution was added, *i.e.*, half saturation with salt.

It is evident that we have here at least four albuminous bodies: dys-albumose, not soluble in water, but soluble in sodic hydrate, which, if it is noted, gave all the reactions in a very marked manner. This was more abundant than the others, owing to the great loss attendant upon their purification; hetero-albumose precipitated by alcohol; and proto-albumose soluble in alcohol and water, but precipitated by half saturation with ammonium sulphate; and the secondary albumoses, precipitated by full saturation with the same salt.

Peptone does not appear, which is rather surprising, since this substance is stated by many to be one of the ultimate products of bacterial action, though contrary to the experience of Fermi and Pampersi, as quoted above. Peptonuria is often stated to result from the action of bacteria in the body and often to indicate the presence of accumulations of pus. Possibly this production of peptone may be a function peculiar only to the pus-forming organisms. The layers do not differ decidedly from each other, except that the middle layer contained practically all the tyrosin, and the lower layer a large amount of a body which must have been hetero-albumose, since it was insoluble in water but soluble in ten per cent sodium chloride solution. This is the substance which falls out of solution when digestive products are dialysed, due to the fact that it requires a certain amount of salt for its solution. The lack of correspondence in the result of the tests applied to the hetero-albumose precipitated by alcohol and that redissolved in the salt solution can be due to nothing else than the paucity of the latter in layers one and two, and its preponderance in layer three. In layer three the dys and hetero albumoses were far in excess of the others, and practically responded to similar reactions.

Some doubt has been expressed whether the albumoses produced by micro-organisms are not more nearly allied to the atmid albumose described by Neumeister, and produced by superheated steam, than to the very similar substance found in gastric digestion; this, too, occurs to me as the more probable, but I am not positive that some of the albumoses produced by the initial treatment of the material may not have been removed, though every effort was made to accomplish this. Salkowski, however, could not separate his atmid albumose into the different varieties, a thing, as seen, which can be readily done with those of bacterial origin.

Blood Fibrin. — The following attempt was made with the fibrin from pigs' blood, 2.7 kilogrammes of which, fresh and well washed, were placed in four liters of water, to which one hundred and twenty cubic centimeters of saturated sodium carbonate solution had been added. This was placed in the brood oven on October sixteenth, and allowed to remain until January sixth. By this time the very offensive odor which at first had been noticeable had gradually disappeared, and in the meantime the fibrin had dissolved, with the exception of a little sediment at the bottom of the open jar in which it had been placed. The greater part was decanted off, the remainder passed through filter paper, and the residue well washed with weak sodium carbonate solution. The fluid was clear, but tinged a reddish brown from the tiny portion of hemoglobin which could not be removed by washing; the whole was then concentrated upon the water bath at a low temperature, the increasing alkalinity being held in check by the addition of weak acetic acid (10 per cent). By this process, a slightly reddish, flaky precipitate was formed, and after a weak acid reaction was established and the liquid boiled for a few minutes, it was filtered. There were obtained seven hundred and twenty cubic centimeters of a clear reddish-brown fluid, whose reaction was again restored to neutral. No reaction could be obtained with nitric acid, but it gave a biuret reaction after decolorization by charcoal. There was then added to the liquid an equal amount of a saturated solution of ammonium sulphate, but no precipitate nor cloudiness was produced, even after standing twenty-four hours. Therefore, hetero and proto-albumose, or Fraction I., according to Pick (Zeit. für physiolog. Chemie, Vol. 24, page 246), were absent. It was then two-thirds saturated with ammonium sulphate, which produced a cloudiness that after twenty-four hours changed into a fine precipitate, but the solution could not be filtered clear, though a considerable precipitate was collected on the filter and purified by solution in water and reprecipitation. The ammonium sulphate was removed by barium hydrate, warming, and filtration, the excess of barium by carbon dioxide and filtration, and the resulting solution, after concentration, used for the subsequent tests. The solution was then completely saturated by ammonium sulphate in substance while warming slightly upon the water bath, which produced a cloudiness that after twenty-four hours became converted into a pasty precipitate at the bottom of the beaker. There was also produced an oily reddish substance, which rose to the surface and was decanted off before the precipitate was removed to the filter. After filtering and washing this precipitate with a saturated solution of the salt, it was redissolved in water and purified as before, after which the salt was removed in the same way. The amount of the material was small, but sufficient for the subsequent tests. The filtrate was poured into concentrated sulphuric acid equal to one-tenth its volume, which had been previously saturated with ammonium sulphate. After twenty-four hours an oily-looking, reddish-brown colored precipitate was formed, which floated on the surface of the fluid, and adhered to the sides of the container. This could not be separated by filtration as it passed through the filter paper. It was removed, however, by a separating funnel, shaken repeatedly with ether, which removed the color, leaving a cloudy residue, which could not be made to separate until water was again added and it was again precipitated with ammonium sulphate in acid solution (one-tenth concentrated sulphuric acid), as before. This time a sticky precipitate was formed, which could be separated, and was purified as before, and used for reactions. The ethereal extract was set aside for further examination. The strongly acid saturated solution was treated with an iodo-iodide of potash solution, also saturated with ammonium sulphate, but only an oily brown colored liquid rose to the surface, which, when separated and shaken with ether, left a colorless solution giving no biuret reaction, from which it was determined that no peptone was present.

The following table expresses briefly the results of the reactions of the different fractions with the reagents commonly employed for albumoses:

	FRACTION II.	FRACTION III.	FRACTION IV.
HNO cold......	Pptd. only with NaCl, clears on heat.	Pptd. only with NaCl, clears with heat.	No change.
NaCl and acetic..	No change.	Cloudiness, clears with heat.	No change.
$CuSO_4$ (1–30)....	No change.	No change.	No change.
Ferrocy. pot. and acetic.........	Cloudiness.	Slight cloudiness.	Cloudiness, no change with heat.
Picric...........	Slight cloudiness.	Slight cloudiness.	Cloudiness, no change with heat.
Tannic..........	Cloudiness with heat unchanged.	Cloudiness.	Cloudiness, clears with heat.
Millon..........	White flakes to red by heat.	No ppt. Turns red on heating.	No ppt. Reddens on heating.
Xantho prot......	Distinct.	Marked.	No change.
Molisch.........	Very marked.	Distinct.	Faint.
Biuret..........	Pale rose. Distinct.	Pale rose.	Faint.
Lead Sub ac. NaOH and heat.	Slight brown ring.	Browning, no ppt.	No change.
Alcohol.........	Ppt. not changed by heat.	Cloudiness, no ppt. Clears on heating.	Cloudiness, clears partly on heating.

These fractions correspond very closely to the three secondary albumoses as isolated from Witte's peptone, in their reactions, with, however, the absence of proto and hetero albumoses. Where reactions fail which were obtained by him, it is unquestionably due to the weaker solutions whose strength could not be absolutely determined on account of the relatively small amounts of the isolated substances employed by me, while Pick's solutions were invariably of a strength of two per cent. The mere fact that no precipitate could be obtained with nitric acid alone, but could with the aid of sodium chloride, together with my inability to obtain a precipitate in the original solution by half saturation with ammonium sulphate, demonstrates to a certainty that the primary albumoses were absent and could not have escaped such half saturation, else they would have been found in fraction II., which Pick calls secondary albumose A. By reference to the results of cooking these fractions with alkali and subacetate of lead, it will be seen that the loosely combined sulphur diminishes with each fraction, until in the last no such body can be found; this also corresponds very closely with Pick's results. It can also be demonstrated that fractions II. and III. contain much more tyrosin, as evidenced by the results of the Millon reaction, while the presence of the reducing body shown by Molisch's test diminishes in the same way. This does not correspond with Pick's results, who found that the carbohydrate was present in all fractions about equally, though in a more recent work (Zur Kenntnis der peptischen Spaltungsproducte des Fibrins, Beitraege z. chem. Physiolog. und Patholog., Vol. II., p. 481), he speaks of the fraction III. as pre-eminently containing the carbohydrate group and calls it glycoalbumose. Here again we find from the readiness with which the albumoses of bacterial origin can be separated by fractional precipitation that they are allied to the digestive products produced by trypsin rather than to the atmid albumoses. As in the previous case, we are also struck by the absence of true peptone, which must be due to the fact that micro-organisms do not produce it, or that on account of the prolongation of the process the peptone once found is converted to amido acids, a proposition that seems untenable on account of the presence of such a marked amount of albumose, which in the natural course of events would also be converted to peptone and to such acids.

Hexone Bases. — This solution containing iodine and saturated with ammonium sulphate was then shaken with mercury and filtered, to remove the iodine, and concentrated until a large portion of the salt was removed, again filtered and barium hydrate added while on the water bath, until all the sulphuric acid was saturated, filtered, the excess of barium removed by carbon dioxide, and the remaining solution treated with bichloride of mercury until acid. By this means a liquid was obtained which must have contained the hexone bodies, if any had been formed by the action of the micro-organisms, which seemed a sufficiently thorough one for this purpose, from the small amount of detritus obtained out of the large quantity of fibrin. The addition of the mercurial salt was, naturally, to isolate the first of these bodies, histidin, according to Kossel's method (Zeit. f. phys. Chemie, Vol. 25, p. 176).

Histidin. — A meager precipitate was formed, which, after being well washed, was suspended in water, treated with sulphuretted hydrogen, the precipitate (mercuric sulphide) removed, the filtrate concentrated to one hundred cubic centimeters and placed upon ice. After fourteen hours a considerable crystalline precipitate was formed, consisting of needles and long four-sided bevelled prisms which showed no tendency to form aggregates, as did the needles. The prisms were soluble in strong hydrochloric acid (33.3 per cent) by which they were dissolved and again precipitated by alcohol-ether mixture (equal parts). They were reprecipitated three times, but this was associated with such great loss that not enough was left for analysis. The needles, when recrystallized from boiling water, proved themselves to be tyrosin, both by the strong Millon's reaction and the amount of nitrogen they contained. The unknown crystals, however, did not correspond at all to the description of Kossel's histidin.

Arginin. — The filtrate from the mercuric chloride precipitate was freed from mercury by sulphuretted hydrogen, the latter driven off by heating, and the whole somewhat concentrated, and, while still hot, silver nitrate in substance (Kossel recommends the sulphate) added until all the chlorine was precipitated, the whole filtered, while hot, from the silver chloride and undissolved silver, and to the cooled fluid barium hydrate in substance was added in excess; the precipitate thus produced was very small in amount and was removed from the filter paper after washing well, suspended in water, the silver removed by sulphuretted hydrogen, the solution concentrated to a small volume and allowed to crystallize. An amorphous and a crystalline substance was formed, the former resulting, no doubt, from the barium, some of which had not been removed, hence it was redissolved by the addition of water and warming and strongly acidulated by sulphuric acid. The barium was now separated as a sulphate, and on re-crystallization after its removal a mass of crystals only was found. These were colorless, consisting of long, narrow, rhombic plates, many arranged in stellate form. The quantity isolated amounted to thirty milligrams, and unfortunately the flask broke while distilling off the ammonia produced by destruction by the Kjeldahl process, hence it could not be identified as arginin.

Lysin.— After the removal of the silver in the filtrate from the above precipitation, and the small amount of barium by a few drops of sulphuric acid, the whole was concentrated on the water bath to a half of its former volume, when there appeared, floating on its surface, a considerable crystalline precipitate, which a microscopic examination showed to consist of spherules, with radial lines. After cooling, this was removed and easily recognized as leucin by the blue dumb-bells and globules produced, when heated with Fehling's solution and allowed to cool. After removal of the leucin, further evaporation showed no tendency to cause the separation of any more leucin, and when the volume had been reduced to about two hundred cubic centimeters, the solution was treated while hot with an alcoholic solution of picric acid and allowed to cool. A precipitate was formed, which was filtured by suction, well washed with alcohol, and recrystallized from hot water. The crystals then appeared quite pure (microscope), consisting of long reddish needles which at 110° gave .20569 grammes. These gave .0112 grammes nitrogen by Kjeldahl, or 5.45 per cent. Lysin picrate demands

18.67 per cent nitrogen, so that very evidently this could not be lysin. On another occasion it would be advisable to remove the tyrosin and leucin before attempting to isolate these hexone bases. From what has preceded, it can be seen that bacterial action on albumoses, even when carried to its extreme, is unable to produce these bodies (hexone), and as Kutscher (Zeitschr f. phys. Chemie, Vol. 25, p. 195) has been able to produce them by means of vigorous tryptic digestion, we may assume that as a splitting agency the action of micro-organisms lies between that of superheated steam and trypsin. Of course more energetic than either is the action of long cooking with hydrochloric or sulphuric acids, which produce the hexone bases from either nucleins or fibrin in fairly large quantities, as has been proven by Kossel, Kutscher, and others.

In conclusion, we may again allude to the results of these efforts as follows: Prolonged action of mixed micro-organisms on nucleins and fibrin, and probably other albuminous bodies, produces proto and hetero albumoses and secondary albumoses A, B, and C, according to Pick, but no peptone. In both respects their action differs from superheated steam, and the albumoses are not atmid albumoses, which cannot be thus separated, but simulate more those produced by trypsin. This bacterial action does not produce hexone bases, and in this respect differs from trypsin, which does. During these investigations there was found a very interesting substance, which presumably was the nitroso-indol described by Salkowski, and my investigations in regard to it will be included in a later communication.

RING BODIES (NUCLEAR REMNANTS?) IN ANEMIC BLOOD.[1]

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The various anomalies in the staining reactions of the red corpuscles occurring in cases of anemia have been for a number of years a familiar feature in writings on the blood. I need only speak of the well-known fact that while normal red corpuscles have a much greater affinity for acid than for basic stains, under pathological conditions this preference for acid stains becomes neutralized or reversed. In specimens stained with any mixture containing both an acid and a basic dye we are accustomed to seeing the normal red corpuscles stain yellow, red, or light brown. When this preference for the acid stain is lost, some of the red cells take up a mixture of acid and basic stains or become actually basophilic. In the majority of cells which are thus "off-color" one sees a diffuse purplish or bluish tint, and to this condition has been given the name of "polychromatophilia."

But at times one finds instead of this diffuse bluish coloration, a spotting or stippling of the red cells with dark blue dots, especially when specimens are stained with basic dyes, such as thionin or methylene blue. To bring out this reaction the ordinary blood stains, of which Ehrlich's triple stain is a type, have been insufficient and one has had, until recently, to go to the trouble of staining especially for these basophilic granulations when their presence was suspected.

One of the great simplifications in blood technic that has come to us with the introduction of Wright's modification of Leishmann's stain[2] is due to the readiness with which this stain brings out all varieties of basophilia, granular or diffuse. The same stain which serves us for ordinary blood work and for the malarial parasite can also be used to bring out all the anomalies of staining reaction characteristic of the severer types of anemia.

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[1] Read before the Boston Society of Medical Sciences, Nov. 18, 1902.

[2] See Journal of Medical Research, January, 1902.

But, more than this, Wright's stain brings out a new set of anomalous staining reactions hitherto undescribed, and it is these which I am concerned with now. The basophilic granulations heretofore described in cases of anemia, especially in those of the pernicious type and in lead poisoning, are ordinarily discrete and not arranged in any special figures. This is shown in Figure 1 which I selected from a number of photographs of the ordinary granular stippling because it has a faint suggestion of a figure-of-eight arrangement. It needs, however, as can be seen, a good deal of imagination to trace this and I show it in order that there may be something with which to contrast the figures which follow. Occasionally, indeed, one notes that the blue-staining granules have a peripheral arrangement (see Fig. 2), but no other definite configuration has been hitherto observed.

I pass now to the figures on account of which this paper was written, the first of which was pointed out to me by Dr. Hewes early last summer in the blood of a case of lead poisoning. As can be seen in Figure 5, we have here a set of granulations arranged distinctly in a circle, with no others inside or outside the circle, and with Wright's modification of Leishmann's stain this ring is stained not blue like the ordinary basophilic granulations, but right red. At one point in the ring (as is often the case) we see a large dot reminding us a little of the nucleus of a malarial parasite. Rings more or less similar to this I have found in three cases of pernicious anemia, three of lead poisoning, and one of lymphatic leukemia. For the most part they are stained red, though occasionally (see Figs. 9 and 10) one meets with a blue stained ring. In Figures 4, 5, 6, 18, and 23 are figured red-staining rings not associated with any stippling of the rest of the corpuscle. In some, for example Figure 4, the ring is very small, corresponding approximately to the size of the nucleus of a normoblast. In others, for example Figure 18, the ring is almost at the periphery of the cell, although a faint rim of corpuscle substance can be traced outside it. In stippled cells (see Figs. 26 and 27) these peripheral rings are not uncommon.

A second class of rings are the relatively small ones occurring in stippled cells (see Figs. 7 to 11). Such rings may be single or made up of several threads (see Figs. 21 and 22 and 24). Occasionally the rings are intertwined in very bizarre fashion (see Figs. 21 to 25 inclusive). In Figure 24 the outlines traced by the ring are not unlike those of the rosette-shaped or clover-leaf nucleus often seen in megaloblasts. The suggestion that these rings represent nuclear remains is still further supported by Figure 17, in which within a large ring we have something more or less suggestive of a nuclear network.

Another group of rings, in some ways the most striking of all, contains the various figure-of-eight-shaped bodies, of which Figure 12 is the best example. Figure 11 shows rather indistinctly a field containing two of these twisted rings and Figure 13 shows a similar figure, apparently extra-cellular. Figures 14, 15, and 16 are further variations of this type. But Figure 15 shows in addition hook-shaped figures which do not correspond with any that I have previously found, either in my own studies or in literature.

The last group, Figures 28, 29, and 30, show rings apparently made up of twisted threads which in Figure 28 are still within a stippled red corpuscle, while in Figures 29 and 30 the corpuscle appears to have fallen away, suggesting that the ring is the most resistant part of the cell.

As to the interpretation of these ring bodies I feel very much in the dark. They are certainly not parasites of any kind; their occurrence in diseases as diverse as lead poisoning, pernicious anemia, and leukemia seems to me to settle this point. That they have some connection with anemia is, I think, equally clear, since in every case in which they have been found a marked anemia has been present. That they do not represent merely accidental configurations of the ordinary granular stippling is shown by two facts:

1. Because they are sometimes continuous and not resolvable into discrete granules.

2. Because they sometimes occur in cells quite free from stippling (see Figs. 4, 5, 6, 18, and 23).

There is no reason to believe them a mark of cellular degeneration; for they are associated most closely with three manifestations of cell-regeneration, viz.:

(*a.*) Nucleated red cells.
(*b.*) Polychromatophilia.
(*c.*) Ordinary granular stippling[1] (blue staining).

That they have some connection with the nucleus formerly possessed by the cell that contains them seems to me not unlikely. Their shapes correspond for the most part very well with those of nuclear figures, and in every blood in which these rings have been found, nucleated red corpuscles have been abundant. The best hypothesis, therefore, that I can suggest to account for these appearances is that they represent nuclear remains; that is, portions of the nucleus especially resistant to the action of whatever forces it is that destroy the nucleus and ultimately the cell itself. The significance of their red color with Wright's stain, a stain which never stains any nucleus red, is not clear to me. I have not been able to stain them with hematoxylon, although the ordinary granular stippling is easily brought out by this pigment.

Have these figures any practical significance? Not so far as I know. I have described them because it seems the safest rule in scientific work to describe everything new that we come across whether we see any significance in it or not.

I am greatly indebted to Dr. Mary Rowley for assistance in studying and in photographing the phenomena above described.

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[1] NOTE. That basophilic granulations in red cells have been considered a sign of degeneration by Ehrlich, Grawitz, and others, I am well aware, but the balance of experimental evidence seems to me to point strongly to the conclusion that they denote unripe, not over-ripe, conditions of the corpuscle. For a full discussion of this controversy, see Schmidt, Experimentelle Beitrage zur Pathologie des Blutes (Fisher, Jena, 1902).

EXPERIMENTS RELATING TO THE QUESTION OF FIXATION OF STRYCHNINE IN ANIMAL TISSUES.*

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When only a toxic dose of strychnine is injected into an animal the subsequent characteristic tetanic convulsions cease after a shorter or longer time without leaving any sequelæ. Why the spasms should not continue indefinitely has always been a subject of various speculations. One of the earliest theories was that strychnine enters into a fixed combination with the tissues or becomes oxidized within the body. Dragandorff,[1] however, proved that strychnine is excreted in the urine without any alteration. It has been further shown by Adam[2] in a case of human poisoning, and was experimentally confirmed by Kratter[3] and Ipsen,[4] that even medicinal doses are traceable in the urine within a few minutes, and that its excretion is completed in less than forty-eight hours.

The recent discoveries of the fixation of toxines by specific body tissues gave an impetus to the revival of the old theory that the animal tissues are capable of fixation also of alkaloids, especially of strychnine. Attempts were made to support this theory by two different experimental methods. One is the mixing of strychnine with some tissues and then injecting it into an animal. Thus Widal and Nobécourt[5] mixed strychnine with the pulp of brain, liver, kidneys, etc., and injected it into animals; they report that the toxic effect was indeed perceptibly impaired. It will, however, be sufficient to mention the experiments of Thoinot and Brouardel[6] who have mixed strychnine with such inert substances as talcum, charcoal, starch, etc., and have observed a retardation in the effect of the strychnine, to show that the factor in

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* Received for publication, Dec. 14, 1902.

these retarding influences is a mechanical one — the impairment of the process of absorption. Another method is that which was introduced by von Czyhlarz and Donath,[7] who tried to prove the theory of the fixation of strychnine by animal tissues by an ingenious experiment. It is chiefly the interpretation of this experiment which engages our attention in this paper. The experiment of von Czyhlarz and Donath, who have published it in 1900, is as follows: After tightly ligating one hind leg of a guinea-pig, they injected a lethal dose of strychnine below the ligature and found that, when the ligature was removed a few hours later, the characteristic effect of strychnine was not forthcoming. They believe the only explanation possible of this observation is that "by a prolonged contact with the subcutaneous tissue, the muscles, and the blood contained therein, the poison becomes in some way fixed or neutralized *in vivo*." Such a conclusion would lead us again to the now familiar idea alluded to before. The idea of toxins being fixed by the cells or fluids of the animal body is now quite a familiar one. Its extension, however, to alkaloidal poisons should not be accepted without repeated experimental confirmation. Soon after the publication of von Czyhlarz and Donath we repeated these experiments[8] on guinea-pigs, animals used exclusively by these authors, and extended them also to more sensitive animals like frogs and rabbits. As to the latter, our results were entirely negative, the prolonged ligation hardly caused even a retardation of the onset of the spasms. In guinea-pigs, an animal which is known to be somewhat refractory to strychnine, the experiment yielded positive results, but only then, when the dose administered was not more than the toxic minimum. For instance, eight milligrams per kilo animal brought out invariably a fatal tetanus.

Judging from these and other experiments, we suggested that the efficacy of strychnine in the above cases was principally diminished, not because the strychnine was fixed by the tissues, but because the absorption was materially impaired in the ligated extremity, so that the strychnine could be carried slowly and in very small doses at a time into the blood, whence it was soon excreted again before it had a chance to build up an efficacious minimum.

Kleine,[9] who soon after became engaged in the same subject, has proven that strychnine appears in the urine even while the constriction is still on. It is, therefore, possible that, owing to the excretion of a fraction of the dose of strychnine in the urine, the dose remaining in the leg when the ligature is removed, is reduced to a sub-minimum one and is consequently ineffective.

Furthermore, there is another essential factor to be thought of. During the long period in which the leg is being constricted the strychnine has an opportunity to thoroughly mix with the blood and lymph of the extremity. Now it is an established fact that strychnine is much less efficacious in a colloid fluid than in a crystalloid. We may add that even for this reason alone the control experiment of von Czyhlarz and Donath is inadequate. When these authors found that strychnine, injected into a ligated extremity shortly before untying the ligature, brought on the characteristic symptoms, we have to remember that the two experiments cannot be compared for the reason that the injected drug in the latter experiment was in an aqueous crystalloid solution and not, as in their primary experiment, in an albuminous colloidal form. In our previous paper we have stated other facts which show the insufficiency of their control experiment.

In the later and more extensive publication of their experiments [10] von Czyhlarz and Donath have not modified their original opinion, although they take notice of our objections in a foot-note, and have even partially confirmed our experiments.

Two theories, then, are offered for the explanation of the surprising result in the experiment of von Czyhlarz and Donath. On the one hand it is assumed that the effective minimum is never reached after the release from the ligature, because: first, a part of the strychnine has been already absorbed during the period of constriction and has been partially eliminated; secondly, the strychnine is absorbed slowly and with difficulty from the ligated extremity because it is suspended there in a colloidal solution, and because many of the blood capillaries and lymphatics have been destroyed and obliterated by thrombosis, etc., after a tight constriction lasting for hours. On the other hand, a new and far-reaching principle is proclaimed, namely: that the strychnine combines or is neutralized *in vivo* by the animal tissues. It seems to us that there should be no difficulty in deciding which interpretation is more simple or more plausible. Ehrlich has repeatedly emphasized, and only recently so,[11] that the alkaloids, being foreign to the animal organism, are not capable of forming with the protoplasm such stable compounds as are known to be formed by toxins. We should also remember that there is no adaptation or immunization for strychnine.

The view of von Czyhlarz and Donath, however, was recently supported by new and thoughtful experiments reported by M. Carrara.[12]

This author repeated successfully the experiment of von Czyhlarz and Donath in nephrectomized animals. In consequence of the double nephrectomy, which operation obliterated the principal path of elimination, Carrara assumes that the poison, even if absorbed very slowly and in very small doses, ought to be retained almost in its entirety within the animal's circulation, and if, nevertheless, the animal fails to manifest any symptoms of strychnine poisoning the failure cannot be due to slowness in absorption, and therefore is due to fixation of the strychnine by the tissues. This argument appears at first sight quite convincing. It rests, however, on a premise that has hitherto not yet been tested by experiment, viz.: the assumption that nephrectomy necessarily leads to a cumulative effect of strychnine. This assumption is not necessarily correct; we know, for instance, that in advanced cases of nephritis urea is excreted in situations where normally not even traces of it are found. Possibly, therefore, a collateral vicarious elimination for other substances may also be established in nephrectomized animals. Guided by this consideration, Meltzer and Salant[13] have studied in an extended series of experiments on nephrectomized rabbits whether subminimum doses of strychnine have a cumulative effect. They came to the following conclusions: if the doses of strychnine are not too large nor injected in too short intervals, such nephrectomized animals will tolerate the gradual injection of the triple lethal dose without exhibiting the least effect of strychnine. The above-mentioned premise, therefore, taken as a matter of course by Carrara, did not stand the test, for subminimum doses had no cumulative effect in nephrectomized rabbits.

Both von Czyhlarz and Donath, as well as Carrara, experimented on guinea-pigs, which, as we have already noted, are much less sensitive to strychnine. We thought it, therefore, necessary to test the premise of Carrara directly on guinea-pigs!

From a large number of experiments we quote here a few abridged protocols:

(1.) Guinea-pig, 420 grms.; double nephrectomy; during the subsequent twenty-four hours injected 7 subminimum doses of nitrate of strychnine, together 3 mgr., equals 7 mgr. per kilo; animal killed two hours after last injection; at no time a trace of hyperesthesia.

(2.) Guinea-pig, 600 grms.; nephrectomy; during 29 hours 8 doses of strychnine, together 5.3 mgr., equals 8.8 mgr. per kilo; no symptoms of strychnine.

(3.) Guinea-pig, 230 grms.; nephrectomy; in 30 hours 8 doses of 0.35 each, together 2.8 mgrs., equals 18 mgr. per kilo; animal died 6 hours after the last injection; never a trace of effect of strychnine.

(4.) Guinea-pig, 300 grms.; nephrectomy; in 48 hours 12 injections of 0.45 mgr., together 5.4 mgr., equals 18 mgr. per kilo; animal had no convulsions, no hyperesthesia, only after the eighth injection there were some transient slight twitchings.

(5.) Guinea-pig, 215 grms.; nephrectomy; in 24 hours 12 doses of 0.31 mgr., together 3.72 mgr., equals 17.3 mgr. per kilo; animal died 11 hours after last injection without any symptoms of strychnine.

(6.) Guinea-pig, 395 grms.; nephrectomy; in 72 hours 13 doses of 0.6 mgr., together 7.8 mgr., equals 19.7 mgr. per kilo; animal died 4 hours after last injection without showing any effect of the poison.

These protocols suffice to prove that we can speak of a cumulative effect of strychnine on nephrectomized guinea-pigs only to a limited degree. We see *that after double nephrectomy quantities of strychnine, far above the fatal dose,* *can be injected without obtaining a single symptom of the characteristic poisoning, provided that we do not use too large single doses, nor that we let them follow each other too closely.* We have injected into nephrectomized guinea-pigs as much as eighteen to twenty milligrams per kilo without causing even a characteristic hyperesthesia. Carrara's experiment, therefore, in which nephrectomized and ligated guinea-pigs received only up to eight milligrams per kilo without a consequent tetanus, are by no means a proof that the strychnine in the ligated extremity was combined with or neutralized by the tissues. We may explain the results just as well by assuming that in consequence of the ligature the absorption of strychnine takes place very slowly and in minimum doses, and that, therefore, no toxic dose is accumulated in the blood.

Thus the explanation of the experiment of von Czyhlarz and Donath remains at the same point which it had occupied before Carrara's experiments made their appearance, viz., that the promulgation of the extraordinary theory of a combination of strychnine with living tissues is not warranted by the results of this experiment.

Our experiments, however, have elicited some other observations which deserve to be recorded, and might even assist in the solution of the problem in hand.

We have established that the toxic minimum is at least not smaller for nephrectomized than for normal guinea-pigs.* On the contrary, in the general run nephrectomized animals seem to require larger doses. Meltzer and Salant[14] made similar observations in rabbits. This is unexpected, inasmuch as with natural elimination checked, we should rather expect to produce a toxic effect with smaller doses. This fact therefore demonstrates also that nephrectomy does not further the cumulative effect of strychnine.

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* Contrary to Carrara, we still believe that four milligrams of strychnine per kilo are not in all cases sufficient to produce convulsions in guinea-pigs; we met with many exceptions, and it would be necessary to use somewhat larger doses if we wish to make controls for von Czyhlarz-Donath's experiments. Moreover, we found that von Czyhlarz himself[15] records experiments (experiment 12, experiment 16, experiment 18) in which he employed four milligrams per kilo without causing more than hyperesthesia.

We might also mention again that in repeating the experiment of von Czyhlarz and Donath we never obtained a positive result with eight milligrams per kilo. These authors themselves employed invariably only six milligrams per kilo; Carrara, however, mentions that he succeeded even with ten milligrams!

This rule, however, does not prevail during the first few hours after nephrectomy. At this time even subminimal doses show a distinct effect in many cases. We have seen animals which succumbed to tetantic convulsions within a few minutes after doses two, two and four-tenths, and two and eight-tenths milligrams per kilo. Such injections should not be made later than three hours after nephrectomy, nor before the animals are entirely out of the narcosis.

Besides marked tetanus some of the nephrectomized animals showed a reaction to subminimum doses of strychnine in a manner which we have never seen in normal animals. Without the development of evident tonic or clonic convulsions there appear slight short jerks either of the whole body or in certain parts which continue almost without remission. On touching the animals one feels a continuous vibration. Soon the animal will lie prostrated with legs extended, nose and belly touching the table, while the vibrations and twitchings persist. Ordinarily these animals succumb within an hour. Every case of tetanus not ending fatally in a short time gradually presents the above mode of reaction. These jerks gave an impression as though the system had not yet developed new ways of ridding itself of the poison, while the absorbed quantity of strychnine was not sufficiently strong to produce regular convulsions or a continuous tetanus. Hence the continuous *sub-tetanic reaction.*

After the last-mentioned observation the question arose whether a slight alteration of Carrara's experiment might not have quite a different result. From Carrara's description we learn that his *modus operandi* was first to perform nephrectomy, then to ligate a leg wherever strychnine was injected. When, under these circumstances, three hours later, the ligature is removed and strychnine gradually begins to enter the circulation, the system may have in the meantime found a way to prevent a cumulative action of strychnine. We have, therefore, changed the order of the several acts of the experiment.

We ligated and injected a limb, and three hours later we performed a double nephrectomy. When, now, after the effect of the narcosis had passed, the ligature is loosened, it might be expected, if the strychnine did not become fixed, that a strychnine effect will be met with, at least occasionally, as at this early stage the system had not yet elaborated its compensatory mechanism for elimination. The slow absorption, therefore, might occasionally lead to a cumulative effect. This expectation was indeed realized. In eleven such experiments we saw, in four instances, a fatal tetanus soon after the removal of the ligature, in two cases sub-tetanic reaction appeared, in two others a distinct hyperesthesia was present. The three remaining animals did not differ from the other nephrectomized guinea-pigs (controls) which had received no strychnine, except in a certain vivacity and greed for food.

It seems to us that the few positive results with fatal tetanus offer an unmistakable proof that in these cases a lethal dose of strychnine was absorbed after the removal of the ligature. In some cases, however, the constriction alters the absorption to such an extent that no sufficient quantity of strychnine can enter the circulation before the final compensatory mechanism is developed; hence the negative results.

Carrara's experiment, therefore, cannot affect our view. On the contrary, its modification proves conclusively that, after the ligature is removed, efficacious doses of strychnine may enter the circulation from the extremity.

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NOTE UPON ERYTHRAGGLUTININS IN A CYST FLUID.[1]

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Bordet (1899) pointed out that theories of agglutination must take account, not alone of bacterial agglutination, but also of the agglutination of blood corpuscles and of casein-particles.

Malkoff (1900) worked upon the agglutination of red blood corpuscles and showed that the clumping substance has specific combining affinity for the element clumped, and, furthermore, in a normal serum which clumps different cells there are as many different specific agglutinins as there are species of cells clumped. Malkoff employed the corpuscles of the goat, against which he immunized guinea-pig and pigeon.

Flexner and Noguchi (1902) studied the effect of venoms upon agglutination, as well as upon lysis, and pointed out that the red corpuscles of the rabbit are highly susceptible, whereas the corpuscles of the guinea-pig, dog, sheep, swine, and ox are less susceptible in that order.

The most recent paper we have seen is that of Ford (1902). Ford chose an agglutinin occurring in normal serum (that in rabbit serum for hen's corpuscles), produced its anti-agglutinin, produced by immunization a second agglutinin of rabbit's serum for hen's corpuscles, and then a corresponding anti-agglutinin. He found that the two anti-agglutinins were interchangeable and therefore identical. He concludes that the body obtained by immunization is merely quantitatively different from the body found normally.

The following notes concern a fluid from a non-malignant

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[1] Received for publication, Dec. 19, 1902.

parovarian cyst removed at operation. The fluid was found to dissolve and to agglutinate the red blood corpuscles of the rabbit. The fluid was heated for one half hour at 54°–59° C., whereupon hemolytic power was lost.

Clumping was obtained of the washed corpuscles of the normal rabbit, of rabbit immunized to the cyst fluid, of dog, and of one man. Other human corpuscles, including those of the patient, were never agglutinated. Agglutination of the guinea-pig's corpuscles was so slow as to be valueless for experimentation.

Upon repeated intraperitoneal injections of the cyst fluid, amounting in all to fifty cubic centimeters, a rabbit was found to yield a serum having the property of delaying or inhibiting the agglutination of its own and other rabbit corpuscles.

The serum of the patient is markedly hemolytic for rabbit corpuscles. Upon inactivation by heat no agglutinative power could be demonstrated.

The fluid (Cy) was prepared by heat for agglutination work (Cy—C, where C is the complement). Saline suspension of washed corpuscles of the rabbit (wcR) was placed in the same amount in a series of test tubes. To each was added a different amount of Cy—C, *e.g.*,

wcR+(Cy—C) gtt. i
wcR+(Cy—C) gtt. ii, etc.

That series of tubes was found in which notable clumping occurred where the observed drop was taken from the hollow of the tube, *e.g.*,

NaCl+wcR+8 (briefly for [Cy—C] gtt. viii),
NaCl+wcR+16,
NaCl+wcR+32.

With this series was taken the limiting negative,

NaCl+wcR+4.

The whole series was centrifugalized. The supernatant fluids, thus,

NaCl+4,
NaCl+8—x,
NaCl+16—x,
NaCl+32—x,

were applied in the same amount to fresh wcR. These supernatant fluids proved to cause agglutination in similar ascending series. Thus, where

$8-x=y$, then
$16-x=y+8$, and
$32-x=y+16$.

The quantities suggested can be only qualitatively confirmed, owing to the nature of the phenomenon of agglutination. Thus

$\mathrm{wcR}+\mathrm{NaCl}+8-x$

gives no reaction, or a border-line reaction, and

$\mathrm{wcR}+\mathrm{NaCl}+16-x <$
$\mathrm{wcR}+\mathrm{NaCl}+32-x.$

SUMMARY.

The foregoing may be summed up as follows:

The cyst fluid exhibits

1. Hetero-erythragglutinins for
    - (*a.*) rabbit.
    - (*b.*) dog.
    - (*c.*) guinea-pig (delayed).
2. Iso-erythragglutinin for one man.
3. No other iso-erythragglutinins in cases examined (no auto-erythragglutinin).

The cyst fluid is capable of developing an anti-erythragglutinin.

The patient's serum, though equally hemolytic with the fluid, shows no agglutinative power.

The rabbit erythragglutinin sustains a quantitative relation to the amounts of corpuscles agglutinated.

A definition of the type of agglutinate obtained in work like the above may serve to bring out more clearly the value of the blood corpuscles in the study of agglutinins. The kind of aggregate observed may be shortly termed poikilagglutinate in contrast with the homoagglutinate or rouleau.

Slight acquaintance with the red corpuscles shows that they clump in two forms, in one of which the arrangement might be simply termed streptic, in the other staphylic. But these terms are scarcely definite enough, as further examination shows.

In the streptic type they adhere rim upon rim to form the coinrows or rouleaux. In the staphylic type they adhere by prominent angles upon surfaces of apposition which are accidental. In the rouleaux the elements preserve to a degree their biconcavity. The corpuscles may remain biconcave in the staphylic aggregates, but they may be variously crenate, vesicular, or otherwise distorted. The elements of a rouleau are alike, whereas the unlikeness in the elements of a tridimensional clump may be striking. Thus the streptic agglutinate and the clump in three dimensions might be more strictly termed, to employ the usual prefixes, homoagglutinate and poikilagglutinate.

Nevertheless, though the elements of a rouleau tend to show biconcavity, the elements of the poikilagglutinate (such as one gets in experiments like the above) may be equally normal-looking. Though the streptic agglutinate may be said to depend upon shape of elements with change of surface or change of ambient fluid, the staphylic agglutinate is formed without regard to element shape, consistently with great variety in surface character of its units, and in the absence of its usual medium.

Striking molar changes in surface, certainly involving marked molecular redistribution, are consistent with the constant and regular occurrence of poikilagglutination. But these changes are so many and so various (crenation, vesicularity, distortion, laking tendency) that they can hardly be invoked as the cause or necessary condition of the clumping.

The phenomena of corpuscular clumping thus serve to demolish in a simple manner those theories of agglutination which regard surface changes as the essence of clumping in general. That demolition had indeed been long since practically accomplished; but the blood corpuscles seem an especially fitting weapon.

The foregoing study emphasizes once more the quantitative character of those phenomena of which hemmagglutination is one representative, sets forth a certain advantage in studying the blood corpuscles to make clear the nature of agglutination, and indicates the desirability of further work upon agglutinins and similar principles of natural occurrence in pathological fluids.

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THE INFLUENCE OF NEPHRECTOMY UPON ABSORPTION.* — AN EXPERIMENTAL STUDY FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH.

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A recent study by us on the effects of subminimum doses of strychnine upon nephrectomized rabbits[1] brought to light the fact that such animals can gradually receive a good deal more than the fatal dose without manifesting any reaction. In the discussion of this remarkable result we suggested, among other explanations, the possibility that in nephrectomized animals absorption from the subcutaneous tissues may be impaired. We have observed, however, in our experiments, that the single minimum toxic dose is the same for nephrectomized as for normal animals, and it appeared to us at first that this fact speaks against the supposed impairment of absorption after nephrectomy, for if under these conditions absorption were retarded the single effective dose ought to be larger in order to obtain here an effect similar to that in normal animals. An analysis of all the factors concerned in this question, however, shows that this fact, far from being opposite to the above-mentioned supposition, might rather be interpreted as favoring the conjecture that absorption is retarded in nephrectomized animals, for we must remember that the effect of an injected dose is not dependent upon absorption alone, but also upon elimination which begins soon after injection. While the absorption of the poison into the blood is slowly going on, its elimination soon commences also, so that the actual effective dose within the blood is not equal to the injected dose, but is represented

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by the quantity absorbed minus the quantity eliminated. In animals without kidneys,[1] that is, with almost no elimination, we might, therefore, expect that the absorption of smaller doses would be sufficient to induce a toxic effect. If we find, nevertheless, that the minimum toxic dose in nephrectomized rabbits is not diminished, we have reason to suspect that the abolition of the elimination is compensated by a retardation of the absorption. We might mention here that in recent experiments on guinea-pigs, Meltzer and Langmann found that the susceptibility to strychnine of their animals is, indeed, increased in the first few hours after nephrectomy.*

However this may be, it is quite clear that the facts which we have observed in our studies of the absorption of strychnine are insufficient to give a positive answer to the question as to whether the power of absorption is affected by nephrectomy. An investigation of this problem, however, seemed to us to be the more desirable since this solution might contribute to the elucidation of the much discussed and still perplexing question of the formation of edema in many cases of kidney disease.

Edema is an abnormal accumulation of lymph within the tissue spaces. The normal quantity of lymph is regulated by the amount of transudation from the blood vessels into the tissue spaces and by the degree of absorption from there into the circulation. Edema, therefore, can be caused either by increased transudation or diminished absorption or by both factors acting together. The prevailing theories of the formation of edema consider chiefly the importance of increased transudation and pay but little attention to the factor of absorption. In the theory of Bright according to which hydremia is the cause of edema, in the theory of Grainger Stewart[2] and of Bartels[3] which lays stress upon the hydremic plethora, or in Cohnheim's[4] theory of increased permeability of the blood capillaries, — in all of these theories it is the increased transudation of lymph into the tissues which is made responsible for the production of edema; and even Lazarus-Barlow,[5] who credits the impaired tissues with an active share in the production of edema, it is the power of the tissues to attract fluid from the blood vessels he thinks of and not their possibly increased power of retaining the fluid once in their possession. As the normal processes of transudation and absorption are to a considerable degree independent of one another, and are continually going on simultaneously, there is theoretically no reason why we could not assume that there might be conditions in which transudation remains normal while absorption is diminished which might then often lead to edema. Landerer's [6] statement that the state of the elasticity of the tissues has an important influence upon the accumulation of lymph within the tissue spaces makes the assumption of the existence of conditions with preferably impaired absorption quite intelligible. Such a theory has, indeed, been brought forward by Grosz and Reichel.[7] Based upon the observations of Landerer that the elasticity of edematous tissues is decreased, Grosz and Reichel assume that in nephritis the tissues become damaged and lose their elasticity, whereby the tension becomes decreased and the lymph is less easily absorbed; hence the gradual formation of anasarca. In support of this theory Reichel [8] brought forward some experimental observations. Normal salt solutions were injected subcutaneously into normal individuals and into patients suffering from heart or kidney disease, and then the time determined at which the diffuse edema thus produced disappeared. Reichel found that while in normal persons the edema disappeared after a few hours, it persisted in patients with cardiac disease for about two days, and in patients with Bright's disease it lasted as long as five, eight, and even ten days. Reichel gained the impression from his experiments that in Bright's disease hardly any absorption takes place. We must say here, however, that Reichel's experiments hardly support his theory. Since it is impossible to study with advantage the effect of subcutaneous injections into tissues that are already edematous, Reichel selected for his experiments such cases of Bright's disease as were not complicated by anasarca, cases of interstitial nephritis, for example. Here is a weak point in

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* See this journal, page 19.

Reichel's argument: if the diminished absorption be, indeed, the essential factor in the formation of edema, why did his patients whose power of absorption was so manifestly impaired not develop any edema?

As to the observations of Landerer, of diminished elasticity in edematous tissue, they might be interpreted that the occurrences of edema within the tissues for any reason whatsoever will cause the loss of their elasticity, but not that the loss of elasticity is the cause of edema. However this may be, it is quite evident that in the discussion of the causation of edema in nephritis the element of absorption has been, indeed, insufficiently studied, and while much experimental work has been done on the factors influencing transudation, there are hardly any experiments on animals bearing upon the question of absorption in its relation to nephritis.

In this paper we record the results of a series of experiments made with a view of determining the behavior of absorption in nephrectomized animals. We shall not enter into a detailed description of our attempts to study the rate of absorption from the subcutaneous tissues; the results have been thus far contradictory and altogether unsatisfactory. Whether this method is capable of giving more reliable results when applied to the human being, as it would appear from the statements of Reichel, is a question we are, for the present, unable to decide. Our attention was soon turned to the study of absorption from the peritoneal cavity, which can be made under conditions capable of giving comparatively precise results. This method, though simple, requires a few remarks. Fluid was introduced into the peritoneal cavity through a minute opening made by an incision in the linea alba which was sutured and clamped immediately after finishing the injection; this is the only method which gives us the assurance that the fluid is introduced into the peritoneal cavity and not into some part of the intestinal canal. Hamburger[9] in his experiments on absorption from the peritoneal cavity introduced the fluid by means of a trocar inserted into the lumbar region. That this method is unreliable was pointed out by Adler and

Meltzer,[10] as the point of the trocar sometimes perforates the gut and all or a part of the fluid enters the lumen of the intestines. Ewald and Schnitzler[11] state that their results were contradictory so long as they introduced the fluid into the peritoneal cavity by means of a hypodermic needle; the autopsies soon taught them that they often injected into the intestines. Bacteriologists and other experimenters employing injections into the peritoneal cavity underestimate the extent of this possible error. Autopsies and the use of staining fluids will demonstrate the correctness of our contention. The recovery of the remaining fluid was accomplished by opening the entire abdomen and turning the animal with the abdomen downward, holding it over a plate. By this method every drop of fluid can be recovered. Hamburger[12] has employed the trocar or a canula also for recovery of the fluid. Adler and Meltzer,[13] Roth,[14] and others have already called attention to the impossibility of removing all fluid by this method. We shall refer to this point again. The rabbits were slightly anesthetized when the fluid was introduced, and were killed or thoroughly anesthetized when the fluid was to be removed.

The kidneys were removed extraperitoneally by the posterior route. The anesthetized animal was placed upon the abdomen and longitudinal incisions made in the back about an inch from the middle line, the fascia split and pushed aside to the margin of the quadratus lumborum, where an entrance into the retroperitoneal space was made. By manipulations on the abdomen the kidney was brought out through the opening, a ligature tightly applied to the *hilus*, the kidney removed, the pedicle replaced, and the several layers of tissue sutured. By this method the peritoneum as a rule remains intact. Once in a while, however, the peritoneum is slightly torn, but the tear is very small and has practically no influence upon the rate of absorption, as we have ascertained by special experiments.

For each experiment two rabbits were employed, which were kept under the same conditions; one was nephrectomized, and the other used as a control; it was not possible to have in each experiment animals of exactly equal size, but the quantity of liquid introduced into the peritoneal cavity was in all cases in proportion to the size of the animal, and amounted usually to about one-twentieth of its weight; the injected liquid was in all cases a solution of sodium chloride of variable concentration. During the interval between the introduction of the solution into the abdominal cavity and the removal of the fluid, the animals were kept in their natural positions in their cages.

We started out, as stated above, with the expectation of finding that in nephrectomized animals absorption is diminished; *i.e.*, that the quantity of salt solution which will disappear from the peritoneal cavity during a given interval will be smaller in nephrectomized than in normal rabbits. According to the accepted view, nephrectomy causes plethora, which, as is assumed, increases the filtration from the blood vessels into the tissues and therefore offers an obstacle to absorption from the tissues into the blood vessels. This agrees with the above recorded experiments of Reichel. Furthermore, there are on record two experiments of Hamburger, from which he draws, indeed, the conclusion that the ligation of the renal arteries retards the absorption from the peritoneal cavity. The first few of our own experiments showed that our expectation was not to be realized, as indicated by the few abbreviated protocols which follow. These experiments were made with solutions of 0.8 per cent of sodium chloride.

Experiment 1a: January 5th, normal rabbit, 1,300 grammes, injected into the peritoneal cavity 75cc. NaCl solution, recovered after 35 minutes 55cc., absorbed 20cc.; *i.e.*, 15.4 per kilo of rabbit, or 40 per cent of the quantity injected.

Experiment 1b: January 5th, nephrectomized rabbit, 1,075 grammes, injected 50cc. immediately after nephrectomy, recovered after 35 minutes 30cc., absorbed 20cc.; that is, 18.6 per kilo of animal, or 60 per cent of the injected quantity.

Experiment 2a: January 15th, normal rabbit, 2,200 grammes, injected 110cc., recovered after 90 minutes 60cc., absorbed 50cc.; that is, 22.7 per kilo of rabbit, or 45 per cent of the injected quantity.

Experiment 2b: January 15th, nephrectomized rabbit, 1,670 grammes, injected immediately after nephrectomy 78cc., recovered after 90 minutes

38cc., absorbed 40cc.; that is, 23.3 per kilo of animal, or 51 per cent of the injected quantity.

Experiment 27a: May 14th, normal rabbit, 3,200 grammes, injected 155cc., recovered after 90 minutes 100cc., absorbed 55cc.; that is, 17cc. per kilo, or 35 per cent of the injected volume.

Experiment 27b: May 14th, nephrectomized rabbit, 3,670 grammes, injected immediately after nephrectomy 185cc., recovered after 90 minutes 114cc., absorbed 71cc.; that is, 19.3 per kilo of animal, or 35 per cent of the injected volume.

Experiment 3a: January 20th, normal rabbit, 1,382 grammes, injected 70cc., recovered after 155 minutes 44cc., absorbed 26cc., or 18.5 per kilo, or 37 per cent of the quantity injected.

Experiment 3b: January 20th, nephrectomized rabbit, 1,370 grammes, injected immediately after nephrectomy 70cc., recovered after 155 minutes 21cc. bloody fluid, absorbed 49cc.; that is, 35 per kilo, or 70 per cent of the injected volume.

Experiment 25a: Normal rabbit, 1,410 grammes, injected 67cc., recovered after 2½ hours 27cc. bloody-looking fluid, absorbed 40cc.; that is, 28.3 per kilo, or 60 per cent of injected volume.

Experiment 25b: Nephrectomized rabbit, 1,800 grammes, injected 90cc., recovered 23cc., absorbed 67cc.; that is, 37 per kilo or 74 per cent of the injected volume. Of this pair of experiments "a" was made in January and "b" in May.

Experiment 4a: January 27th, normal rabbit, 1,300 grammes, starved for 27 hours, injected 80cc., recovered after five hours 30cc. (fluid tinged red), absorbed 50cc.; that is, 38.5 per kilo, or 60 per cent of the injected volume.

Experiment 4b: January 28th, nephrectomized rabbit, 950 grammes, injected 27 hours after nephrectomy 60cc, removed after five hours 20cc., absorbed 40cc.; that is, 42 per kilo, or 66 per cent of the injected volume.

Experiment 26a: May 14th, normal rabbit, 1,920 grammes, starved 27 hours, injected 94cc., recovered after five hours 51cc.; that is, 26 per kilo, or 45 per cent of the injected volume.

Experiment 26b: May 14th, nephrectomized rabbit, 1,350 grammes, injected 27 hours after double nephrectomy 68cc, recovered after five hours 36cc.; that is, 23.7 per kilo, or 47 per cent of the injected volume.

Experiment 5a: February 4th, normal rabbit, 1,610 grammes, injected 100cc.; after 19 hours only a few drops of bloody fluid could be obtained.

Experiment 5b: February 4th, nephrectomized rabbit, 2,060 grammes, injected immediately after nephrectomy 120cc.; after 19 hours not a drop of fluid could be obtained.

It is obvious that a simple comparison of the amounts absorbed in the different animals cannot serve as a basis for comparing the power of absorption in these animals. From G. Wegner[15] and other investigators we know that the size of the animals as well as the quantity introduced into the abdominal cavity have a determining influence upon the rate of absorption. The following table (Table I.) presents the rates of absorption in all the experiments made with 0.8 per cent sodium chloride and computed to per kilo animal as well as in percentages of the injected quantity of the solutions. The horizontal columns give the data pertaining to each pair of experiments; in the vertical columns the first gives the number of the experiment; the second column the time of the injection of the solution after nephrectomy; the third the time the solution remained in the peritoneal cavity; the fourth (pair of columns) give the absorption per kilo in the normal and the nephrectomized animal, and the fifth (pair of columns) the absorption is given in the normal and the nephrectomized animals in percentages of the injected salt solution.

TABLE I.

Showing the rate of absorption in the experiments made with injections of solutions of 0.8 per cent sodium chloride into the peritoneal cavity of normal and nephrectomized rabbits.

The experiment number.	When injected after nephrectomy.	Time solution remained in peritoneal cavity.	Absorption per kilo.		Absorption in per cent of volume.	
			Normal.	Nephrec.	Normal.	Nephrec.
1	Immediately	35 minutes	15.4	18.6	40%	60%
2	"	90 "	22.7	23.3	45%	51%
27	"	90 "	17	19.3	35%	38%
3	"	155 "	18.5	35	37%	70%
25	"	150 "	28.3+	37	60%	74%
4	27 hours	5 hours	38.5+	42	60%	66%
26	27 hours	5 "	26	23.7	45%	47%
5	Immediately	19 "	All	All	All	All

+ indicates that the recovered fluid was more or less bloody.

This table demonstrates at a glance that normal animals certainly do not show a better absorption than nephrectomized ones. On the contrary, in each of the first five experiments the rate of absorption is more or less markedly greater in the nephrectomized animal than in the corresponding normal controls, and even extending our controlling comparisons to animals of different experiments we find only one instance in which the absorption in the normal animal of one experiment is a trifle better than the absorption during an equal interval in a nephrectomized rabbit of another experiment. The absorption in the normal rabbit of Experiment 2 is, after a stay of twenty minutes, twenty-two and seven-tenths cubic centimeters per kilo, while in the nephrectomized, in Experiment 27, the amount absorbed during an equal period was only nineteen and three-tenths cubic centimeters. This latter experiment was made in the month of May, while Experiment 2 was performed in January, and judging by our experience we have reason to believe that the absorption during the summer months is apparently less vigorous than in the winter; the absorption of the nephrectomized animal of Experiment 27 can therefore be compared only with its own control, and not with that of Experiment 2.

We are fully aware, however, that the comparison between single experiments does not permit as yet of the drawing of any positive conclusions regarding the rate of absorption. In a series of experiments by Adler and Meltzer on absorption from the peritoneal cavity in normal rabbits, the writers found that the rate of absorption shows considerable variation in individual rabbits, so much so, indeed, that in two rabbits they recovered after forty minutes even more than they injected. On the other hand, the same authors state that the variability was confined to a small number of experiments, and that in the majority of cases the rates of absorption of solutions of the same concentration do not differ widely. For solutions of 0.75 per cent sodium chloride which Adler and Meltzer[16] mostly employed, thirty-five to forty cubic centimeters was about the average quantity which was absorbed in forty minutes. For a concentration of 0.92 per cent the quantity absorbed was distinctly smaller, and for hypotonic solutions of 0.6 or 0.3 per cent the quantities were unmistakably larger. The authors say further, that notwithstanding the variability of absorption existing for every degree of concentration, the difference of the rates of absorption for the different concentrations comes easily to light in any series of experiments.

In our series of observations with 0.8 per cent solution the number of experiments with each interval (35, 90 minutes, etc.) was comparatively small, and the possibility is not excluded that the apparently greater rate of absorption in the nephrectomized animals was only accidental, and that a larger series of experiments might perhaps show entirely different results. The probability, however, is that this increase in the absorption in nephrectomized rabbits was not a mere accident, and this is well supported by the fact that the same result came out not only in every experiment of the same interval, but also in all the different intervals except when the solution remained for five hours in the peritoneal cavity. We shall see later that just this exception rather strengthens the validity of the rule. The superiority of the absorption in the nephrectomized rabbits becomes more pronounced when the results of the first five experiments are added together; the ratio is then per kilo 101.9 for all the five normal and 133.2 for the five nephrectomized rabbits, or 20.38 per kilo normal and 22.6 per kilo of a nephrectomized animal for each individual experiment. The difference is still more striking when it is presented in percentages of the injected volumes; it is 217 per cent for the five normal and 328 per cent for the five nephrectomized animals, or the ratio for each individual experiment is 43.4 per cent to 65.6 per cent!

Our assumption that in nephrectomized animals absorption is diminished was based, as stated above, on the generally accepted supposition that the removal of the kidneys leads to plethora, which in turn is the cause of an increased power of filtration through the walls of the blood vessels into the lymph spaces, including the serous cavities. Desiring to test this effect of filtration it was necessary, as much as possible, to exclude other factors influencing absorption — in the first place, the factor of osmosis. We have, therefore, started our experiments with solutions of 0.8 sodium chloride, on the supposition that this concentration is isotonic, or nearly so, with the blood and lymph of the rabbit. We arrived at this assumption through a study of the results of Heidenhain and Orlow,[17] who introduced solutions of sodium chloride of various concentrations into the peritoneal cavity of normal dogs, removed the remainder after a few hours, and then tested their concentration. They found that no matter whether the original fluid was hypotonic, isotonic, or hypertonic, the final concentration of the fluid recovered was almost the same, always nearly 0.73 per cent. Hamburger[18] arrived at the same conclusion; that is, he also found that solutions of different concentrations, after remaining for a couple of hours in the peritoneal cavity, assumed the same final osmotic pressure, which was, according to Hamburger, equal to that of the blood serum of the animal. Although Hamburger considers a concentration of 0.94 per cent sodium chloride as isotonic with the blood serum, we had, for reasons we do not care to discuss here in detail, more confidence in the figures of Heidenhain and Orlow and, therefore, at the outset of our experimentation compromised upon the figure of 0.8 per cent as being at least very nearly isotonic with the blood serum of the rabbit.

After having obtained and analyzed our results with 0.8 per cent sodium chloride which indicated the great probability that in nephrectomized rabbits absorption instead of being less active is rather more so than in normal ones, we were led to the following consideration: The idea occurred to us that for the reasons for which we assume that the removal of the kidneys causes an increase in the quantity of the blood, for the same reasons we might also expect that double nephrectomy might be followed by an increase of the osmotic pressure of the blood serum. The osmotic pressure of the urine is normally higher than that of the blood serum, the blood gets rid through the kidneys of a crystalloid solution which is more concentrated than its own serum. When, therefore, this outlet for a surplus of salts — the kidneys — is removed, the blood is compelled to retain a larger amount of salts, and we ought to find that the osmotic pressure of the blood is increased. Now if this be the case, that is, if the osmotic pressure of the blood of a nephrectomized animal is indeed higher than that of a normal one, then there is a new factor in the blood of the nephrectomized animal which is capable of favoring absorption from the lymph spaces into the blood. Assuming that a solution of 0.8 per cent sodium chloride is isotonic with the blood of the normal rabbit, this solution ought, then, to be hypotonic with the blood of the nephrectomized animal and, therefore, more readily absorbed. On this basis we can further understand why in the two experiments (4 and 26) in which the solutions were kept for five hours in the peritoneal cavities the differences in the rates of absorption nearly disappeared. As we have already seen above, all the solutions of whatever concentration after a stay in the peritoneal cavity for a few hours assume an equal osmotic pressure which is presumably isotonic with the blood serum. The factor of increased osmotic pressure, which is the cause of the absorption in the nephrectomized animal, therefore, disappears after a few hours, and the rates of absorption become equal in both sets of animals, or the absorption in the normal animal might now be even favored by the still persisting factor of increased filtrating power of the blood.

In the experiments of Adler and Meltzer[19] it was found that the increase in the concentration of the solution from 0.75 per cent to 0.92 per cent caused in proportion a much greater falling off in the rate of absorption than the increase in the hypotonic solutions from 0.3 to 0.6 per cent. We had, then, reason to assume that by increasing the concentrations of the injected solutions the difference in the rate of absorption between the nephrectomized and the normal rabbits would gradually diminish. On the other hand, by employing a variety of stronger solutions, we might thus find a concentration which would still be isotonic for the nephrectomized animal, and would, therefore, be absorbed, while it would be hypertonic for the normal and would, therefore, during the short stay not be absorbed at all; or the solution might even become increased, as it was casually observed by Heidenhain and Orlow[20] in their experiments with hypertonic solutions. Furthermore, from our new point of view, namely, that the nephrectomy is the cause also of an increased osmotic pressure of the blood serum, we had to test the influence of the time elapsing between nephrectomy and the injection of the solutions into the peritoneal cavity, as it was reasonable to expect that the sudden difference in the osmotic pressure between the blood serum and the lymph of the tissues will gradually diminish and assume perhaps relations as they exist in the normal animal. The nearly even conditions noticed in Experiments 4 and 26 in the previous table might have been due as well to the long stay of the solutions in the peritoneal cavity (five hours) as to the long interval (twenty-seven hours) which passed between the nephrectomy and the injections of the solution. With the new hypothesis and the just mentioned point in mind, our further experimentation was continued with hypertonic solutions of sodium chloride of various concentrations. These were injected into the peritoneal cavity either immediately after nephrectomy or many hours later, and were recovered, as in the previous experiments, after a stay of variable intervals. We shall not describe these experiments in detail, but present them only in short tables.

Table II.

Showing the rates of absorption in a few experiments made with one per cent solutions of sodium chloride.

Experiment number.	When injected after double nephrectony.	Solutions remained in peritoneal cavity.	Absorption per kilo.		Absorption in per cent of injected vol.	
			Normal	Nephrec.	Normal	Nephrec.
6	19 hours	165 minutes	36.3+	38.5+	72%+	77%+
38	25 "	160 "	22.5	21.4	46%	43%
7	22 "	90 "	8.5	23.7	18%	47%
30	27 "	90 "	—29.	22.	—60%	43%

All the experiments with higher numbers were made during the summer and show a lower absorption.

In Experiment 6 the cross indicates here, as in the other tables, that the fluid recovered was bloody; this occurred in normal as well as in nephrectomized rabbits.

In Experiments 6 and 38, in which the slightly hypertonic solution was injected long after nephrectomy and remained nearly three hours in the peritoneal cavity, there is practically no difference between the normal and the nephrectomized animal.

In Experiments 7 and 30 in which the solutions remained in the abdominal cavity only ninety minutes, the absorption was distinctly better in the nephrectomized than in the normal rabbit. The minus in Experiment 30 means that more fluid was removed than injected. There was an increase of fluid within the normal animal to about sixty per cent of the amount injected, while in the nephrectomized animal about forty-three per cent of the injected fluid was absorbed.

TABLE III.

Showing the rates of absorption in experiments made with one and two-tenths per cent solutions of sodium chloride.

Experiment number.	When injected after nephrectomy.	Solution remained in peritoneal cavity.	Absorption per kilo.		Absorption, per cent of volume.	
			Normal.	Nephrec.	Normal.	Nephrec.
8	21 hours	30 minutes	3·9	7·3	8%	15%
9	17 “	65 “	—3.0	16.3+	—5.6%	33%+
39	Immediately	90 “	5·5	13.8	11%	30%
41	“	90 “	—18	3.6	—40%	7·5%
10	20 hours	90 “	††18	31+	36%	63%+
11	22 “	90 “	7·0	15·5	14%	32
12	21 “	90 “	4·5+	20	10%+	40
13	24 “	90 “	3·3	10	6.6%	20
33	41 “	90 “	—6.4	16.0	—12%	30%
34	41 “	90 “	10	10	20%	23%
14	22 “	3 hours	15·5	23+	37%	48%+
15	22 “	6 “	31.2	32.6	62%	64%

+ Means recovered fluid looks bloody.
†† Clamp fell off and some fluid probably escaped.

This table gives us interesting information. In the first ten experiments, in which the solution did not remain in the peritoneal cavity longer than ninety minutes, there were in the normal animals three with negative absorption, which means that more fluid was recovered from the peritoneal cavity than was injected, against none in the nephrectomized ones. Furthermore, if we leave out from this series of experiments number ten (No. 10), in which the absorption in the normal could not be established accurately (the clamp fell off and some fluid was lost), and leaving out also Experiments 33 and 34, in which the solutions were injected forty-one hours after nephrectomy, we have a series of seven experiments in which the solutions in the peritoneal cavity remained not longer than ninety minutes, and in all of which the absorption in the nephrectomized animals is distinctly better than in the normal one. If we add the results of each of these seven experiments together, the difference between the normal and the nephrectomized animals is, indeed, very striking. The sum total of the absorption per kilo in the seven normal rabbits amounts only to three and two-tenths cubic centimeters, while in the nephrectomized it amounts to eighty-six cubic centimeters, which means for each animal 0.45cc. per kilo for the normal to 12.3cc. per kilo for the nephrectomized rabbit. And summing up the percentages of the injected volumes, it brings out a proportion of 4 per cent to 177 per cent, or 0.57 per cent to 25 per cent. In the experiments made with 0.8 per cent of sodium chloride the average per kilo for the nephrectomized animals was 22.6cc. and for the normal ones 20.38cc., and the average in percentages of the injected volumes was, for the nephrectomized 65.6 per cent and for the normal 43.4 per cent. In other words, the quotient of the increase of absorption in favor of the nephrectomized animal was per kilo 22.6 to 20.38 = 1.1, and in percentage of volumes injected 65.6 to 43.4 = 1.51. In the experiments of this table (Table III.), made with a solution of one and two-tenths per cent, the quotient of increase of absorption in favor of the nephrectomized animals is per kilo 12.3:0.45 = 27.5, and in percentage of the volumes injected 25:0.57 = 43.15. That demonstrates clearly that in using solutions of one and two-tenths per cent the increase in the absorption in favor of the nephrectomized animal is distinctly larger than that which occurs when solutions of eight-tenths of one per cent were employed, the proportion being per kilo of animal 27.5:1.1, and in percentage of volume 43.15:1.51.

Furthermore, in five of these seven experiments (of Table III.) the solution was injected into the peritoneal cavity late after nephrectomy — seventeen to twenty-four hours. This had apparently no influence upon the great readiness of absorption in the nephrectomized animals. We, therefore, learn the interesting fact that the conditions which favor absorption in the nephrectomized animal are apparently still in undiminished force twenty-four hours after nephrectomy.

In Experiments 33 and 34 the attempt was made to introduce the solution of one and two-tenths per cent into the peritoneal cavity forty-one hours after nephrectomy. In Experiment 34 there is no difference between the normal and the operated animal; but in Experiment 33 there is a distinct difference in favor of the nephrectomized animal. It did not seem to us advisable to try on rabbits a still longer interval, as they survive double nephrectomy usually not much longer than forty-five to fifty hours. Besides, a diminished absorption so long after nephrectomy might be due to diminished vitality, impaired circulation, and so forth, and not at all to changes in the osmotic pressure of the blood serum.

TABLE IV.

Showing the rates of absorption in experiments made with solutions of one and one-half per cent sodium chloride.

No. of experiments.	When injected after nephrectomy.	Solutions remained in per cent.	Absorption per kilo.		Absorption per cent of injected volume.	
			Normal.	Nephrec.	Normal.	Nephrec.
18........	22 hours	90 minutes	—9.4	7.7	—19%	16%
19........	24 "	90 "	—10	7	—12%	30%
16........	20 "	3 hours	—4.9	23+	—10.5%	46%+
17........	18 "	3 "	8	8.5	16%	17%

In this short table of experiments with one and one-half per cent solution of sodium chloride we see that in three out of four normal animals more fluid was recovered than was injected, while in all the nephrectomized animals the absorption was positive. As to the rates of absorption, the number of experiments was too small to permit of the drawing of positive conclusions. However, there seems to be no material difference in the relations between the rates of absorption in these experiments and those observed in the experiments made with solutions of one and two-tenths per cent.

Table V.

Showing the rate of absorption in experiments made with two per cent sodium chloride.

No. of experiments.	When injected after nephrectomy.	Solutions remained in per cent.	Absorption per kilo.		Absorption per cent of injection volume.	
			Normal.	Nephrec.	Normal.	Nephrec.
31........	21 hours	90 minutes	—12.5	— 6.0	—26%	—12%
32........	20 "	90 "	—17.5	— 2.5	—39%	— 5%
20........	20 "	5 hours	5	5	11%	10%
21........	20 "	5 "	10+	10	23%	20%+
23........	21 "	5 "	0	36	0%	74%
24........	2 "	17 "	52.1	47.2	81%	80%

In this last table of experiments with the hypertonic solution of two per cent, we find in the two experiments, thirty-one and thirty-two, in which the solution remained in the peritoneal cavity ninety minutes, that in nephrectomized rabbits also more fluid was recovered than injected, but the increase in the fluid is distinctly less than in the normal ones. This is still in harmony with our results in the experiments with solutions of lesser concentration.

Of the three experiments with a stay of the hypertonic solution in the peritoneal cavity for five hours (Nos. 20, 21, and 23), in two there was practically no difference between the normal and the nephrectomized animals, which agrees with the results in the two experiments made with a solution of eight-tenths per cent (Experiments 4 and 26, Table I.) and Experiment 15 (in Table III.) (six hours) with a solution of one and two-tenths per cent. Experiment 23, with no absorption in the normal and considerable absorption in the nephrectomized rabbit, is, indeed, an exception. In experiments of Heidenhain and Orlow[21] with hypertonic solutions of two per cent sodium chloride in normal dogs, there occurred also single exceptions which are entirely out of proportion to what was found in the majority of experiments.

Experiment 24 is likewise instructive. With isotonic solutions we usually find after a stay of seventeen hours in the peritoneal cavity that all is absorbed. With a hypertonic solution of two per cent we find here, after seventeen hours, a part not yet absorbed, but the degree of absorption is almost the same in both normal and nephrectomized animals.

We have to record yet one more observation which appears to us to be significant. In many normal rabbits when the abdomen is opened a larger or smaller amount of lymph comes into view. The amount of normal lymph is of course a function of the two opposing factors: transudation and absorption. Transudation—absorption=lymph. The mutual relations are, it seems, normally variable, though as a rule only within narrow limits. It is this fact which makes the rates of absorption from the peritoneal cavity in the normal animal variable, and which causes the appearance of a variable amount of lymph in the normal peritoneal cavity. Now in none of the nephrectomized animals was lymph ever found on opening the abdomen. The interpretation of this phenomenon is apparently this: The nephrectomy causes immediately an increase of the power of absorption from the lymph spaces into blood. This increased absorption removes any surplus of lymph in the abdominal cavity during the short time which elapses between the nephrectomy and the opening of the abdomen. Our experiments have then brought out the following facts:

1. In nephrectomized rabbits absorption from the peritoneal cavity was found to be more active than under normal conditions.

2. This increase in absorption was most marked in experiments with solutions of sodium chloride of one and two-tenths per cent, which is decidedly hypertonic for normal animals.

3. In experiments with solutions of one and five-tenths per cent, or of less concentration, not a single case in nephrectomized rabbits occurred in which there was an increase of the fluid injected into the peritoneal cavity, while in normal rabbits such an occurrence is not infrequent,

especially when hypertonic solutions are employed, be they even as slight as one per cent of sodium chloride.

4. The increase of absorption in the nephrectomized rabbits continues to be manifest even when the solutions are introduced into the peritoneal cavity about twenty-four hours after nephrectomy. We have reason, however, to surmise that at about a period of forty hours or more after the nephrectomy the factor favoring absorption commences to diminish.

5. When a solution remains in the abdominal cavity five hours or longer the rates of further absorption of this solution seem to become the same for nephrectomized as for normal animals.

6. In none of the nephrectomized rabbits was any lymph found on opening the abdomen.

In view of these facts we accept the above mentioned hypothesis which was the starting point for our experimentation with hypertonic solutions. We assume that with the removal of the kidneys the osmotic pressure of the blood becomes increased, and to such a degree as to more than counterbalance the effect of the simultaneously increased blood volume which might be the cause of an increase of the power of filtration from the blood into the tissues. From our experiments we have reason to surmise that the osmotic pressure of the blood of nephrectomized rabbits corresponds to the osmotic pressure of a solution of sodium chloride of a concentration somewhere midway between one and two-tenths or one and five-tenths per cent. In looking over the literature we found two casual brief statements regarding the osmotic pressure of the blood of nephrectomized animals. One statement is made by von Limbeck,[22] who claims that the isotonic condition of the blood remains unchanged after nephrectomy. He supports his contention by a single experiment made upon a dog. He tested the osmotic pressure of the blood of this animal first shortly before nephrectomy and then again just before the death of the animal—"Knapp von dem Tode"—and found no difference between the tests.

As the second test was made more than forty hours after nephrectomy, the animal apparently being already in a dying condition, this single observation can have no bearing upon our result.

The second statement is made by A. von Koranyi,[23] who made the observation that in rabbits the osmotic pressure of the blood increases considerably — "geht gewaltig in die Höhe" — after the removal of the kidneys (page 41, footnote); after double nephrectomy in rabbits the lowering of the freezing point increased after three hours to Δ 0.61°, and after seven hours to Δ 0.73°; an increase from Δ 0.56° for the normal to Δ 0.73° is equal to the pressure of a column of water twenty and nine-tenths meters high. This statement is, of course, in direct harmony with the assumption which we have made in interpreting our results.

These results were not anticipated by ourselves and are contrary to the expectations and à priori opinions of many writers. We shall, however, not enter upon a discussion of these opinions, neither shall we attempt to compare our results with the above mentioned statements of Reichel,[25] according to which patients suffering from nephritis show hardly any absorption from the subcutaneous tissues, for our observations so far refer only to the behavior of absorption in the animal during the first twenty-four hours following nephrectomy. We have, however, to enter into a discussion of the above mentioned experiments of Hamburger,[24] whose results are diametrically opposed to our own.

This author made two experiments with the ligation of the renal arteries in rabbits and the injection of a salt solution into the peritoneal cavity. He draws the conclusion that absorption takes place also after nephrectomy, but not as rapidly as under normal conditions. In both experiments twenty-five cubic centimeters of blood were withdrawn from the animal, one hundred and fifty cubic centimeters of a two per cent salt solution was then injected into the peritoneal cavity, and later removed. The osmotic pressure of the serum as well as of the peritoneal fluid was tested in the first experiment by the red-blood-corpuscles method of

Hamburger, and in the second experiment by the lowering of the freezing point method. The red-blood-corpuscles method consists in establishing the amount of water which must be added to two and one-half cubic centimeters of serum containing one drop of blood before the first sign of hemoglobin appears in the serum. The first experiment reads as follows: Renal arteries tied, twenty-five cubic centimeters of blood removed, one hundred and fifty cubic centimeters of two per cent sodium chloride injected; recovered after an hour one hundred and thirty-one cubic centimeters, absorbed nineteen cubic centimeters; of the recovered solution one hundred and ten cubic centimeters was again injected, and after one hour eighty cubic centimeters were found, absorbed thirty cubic centimeters. The osmotic pressure of the serum before the injection of the solution into the peritoneal cavity was equal to one and nine-tenths cubic centimeters water, after the second removal it was equal to four cubic centimeters water. The osmotic pressure of the salt solution was before injection equal to seven cubic centimeters of water; after the first recovery it was equal to four and four-tenths cubic centimeters water, and after the second recovery it was equal to four cubic centimeters water.

The second experiment was as follows: Renal arteries tied, twenty-five cubic centimeters blood removed, injected intravenously fifty cubic centimeters three per cent sodium chloride, injected into the peritoneal cavity one hundred and fifty cubic centimeters of two per cent sodium chloride solution, recovered after an hour one hundred and forty-eight cubic centimeters, absorbed two cubic centimeters, injected again one hundred and twenty-five cubic centimeters of the same solution, recovered an hour later ninety-three cubic centimeters, absorbed thirty-two cubic centimeters. Osmotic pressure of the blood serum before injection into the peritoneal cavity $\Delta = 0.556$, after the second recovery $\Delta = 0.701$.

Hamburger made no special control experiments, but he had for comparison a single experiment in another series in which an injection of two per cent sodium chloride solution was made into a normal rabbit. In this case there was indeed a somewhat greater absorption than in the other two experiments. From these experiments Hamburger draws the conclusions, as stated above, that the absorption in nephrectomized animals is reduced. As to the increase of osmotic pressure of the blood serum, Hamburger ascribes that in the first experiment to the absorption of salt by the blood from the peritoneal cavity, and in the second experiment also, of course, to the injection of the concentrated salt solution directly into the blood. Let us now see the validity of Hamburger's conclusions:

1. Hamburger recovers the fluid from the peritoneal cavity by a trocar introduced through the lumbar region into the abdominal cavity. Adler and Meltzer,[26] as well as Roth,[27] have already called attention to the impropriety of this method, by which it is absolutely impossible to say how much remains unabsorbed, being retained between the intestinal coils in the abdominal cavity. The quantities which are absorbed in many of Hamburger's experiments are, therefore, much too large. Hamburger's method certainly does not permit of any comparison of one experiment with another.

2. Hamburger based his conclusions upon only two experiments with nephrectomy and had for comparison only one experiment on a normal animal. The rates of absorption in the two nephrectomized as well as in the one normal rabbit might accidentally have been the exceptions which, as we have seen, are plentiful in a long series of observations. One is not justified in drawing conclusions from only two experiments and one control.

3. Strong hypertonic solutions of two per cent sodium chloride are not suitable for the study of the differences of absorption as they irritate the peritoneum and cause hyperemia which influences the rate of absorption; besides, the crystalloid solution soon becomes converted into a considerably albuminous fluid which follows other laws of absorption than pure salt solution. We should not be justified

in adopting our own conclusions if we had to derive them only from experiments made with hypertonic solutions of two per cent sodium chloride.

4. To study the effect of nephrectomy upon absorption it is certainly inadvisable to introduce in the experiment such factors as the withdrawal of blood or the intravenous injection of three per cent solutions of sodium chloride which by themselves can influence the rate of absorption.

5. Hamburger has never studied the behavior of the osmotic pressure of the blood after nephrectomy and without injecting strong hypertonic solutions into the peritoneal cavity or into the blood or into both. His contention, therefore, that the increase of the osmotic pressure of the blood in his experiments was due solely to the injection of strong salt solutions is not necessarily correct. It apparently did not occur to Hamburger that the removal of the kidneys alone could cause an increase in the osmotic pressure of the blood. As we have reported above, Koranyi found the osmotic pressure of the blood in a rabbit seven hours after nephrectomy to be $\Delta = 0.73$, which is higher than that given by Hamburger in his second experiment of nephrectomy with intra-peritoneal injections of one hundred and fifty cubic centimeters sodium chloride and intravenous injection of fifty cubic centimeters three per cent sodium chloride solution.

For all these reasons we believe that these statements of Hamburger can be neglected in a consideration of the behavior of absorption in nephrectomized animals. We have entered into a detailed discussion of these statements because the two experiments of Hamburger are the only ones which have been made on the subject of our studies, and because they come from an investigator who made valuable contributions to medical science, whose statements, therefore, merit a careful analysis before a disagreement with his conclusions. However, it is due to Hamburger, as well as to our own results, to state that Hamburger was apparently more interested in the proof that some absorption continues to take place in nephrectomized animals than to insist that absorption is less in these animals than in normal ones.

To summarize briefly: The chief result of our long series of experiments is the bringing to light and the establishing of the unexpected and important fact that the removal of the kidneys instead of decreasing rather increases the absorption of fluid from the tissues into the circulation, which is apparently due to an increase in the osmotic pressure of the blood serum. This increase continues in full force for at least twenty-four hours, and lasts probably a good deal longer, although with a gradually diminishing intensity.

In the studies of the factors concerned in the development of edema, experiments with double nephrectomy can therefore be apparently of very little assistance. The animal does not survive long enough to reach a period in which absorption might be indeed diminished, while in the period following immediately after nephrectomy the increased absorption will directly prevent the formation of edema. The latter fact explains in a simple way the well-known experience that no edema appears soon after double nephrectomy, an experience which has been always a puzzle to many investigators. Now also we can understand the absence of edema in prolonged cases of anuria either of hysterical or reflex origin, or due to a simultaneous impaction of the ureters by calculi or by the invasion of neoplasms as they were mentioned by Cohnheim[28] and others. The first effect of anuria is not an increase of lymph in the tissues, but rather an increase of its absorption from the tissues into the circulation, thereby preventing the formation of edema.

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ON FATTY DEGENERATION.

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Through the researches of Pflueger and his students we know that all of the fat in tissues cannot be extracted by means of a simple fat-solvent such as ether or petroleum ether. This resistance to extraction on the part of a portion of the tissue-fat is not a mechanical or a physical phenomenon. The tissue may be pulverized to the finest consistency, it may be extracted in the most varied ways and through the greatest length of time; a portion of the fat cannot be extracted. When, however, the tissue is subjected to some procedure which alters its chemical state, the entire fat may be easily extracted. Digestion by means of pepsin was the method ordinarily employed by Dormeyer; there are, however, many methods which are equally effective, so far as the setting free of the fats is concerned. A hot digestion in weak alkali, as in the Kuelz method for the estimation of glycogen, will set free all the fat, though not in the preformed state. A pancreatic digestion will accomplish the same result, though here also the fats are split to a greater or less extent. Trypsin, in a pure state, would probably free the fats without affecting them; but in all the specimens of commercial trypsin I have used there has been some fat-splitting action displayed. Papain will free the fats, though less effectively than pepsin. The obvious interpretation of these facts is that while the larger part of the fat present in tissues is in the form of free fat, a portion is in combination with certain of the tissue constituents — in all probability with protein. Analogous combinations with tissue constituents are known to exist for sugar and glycogen. When the tissue is properly dehydrated, pulverized, and extracted with ether or ligroin in a proper manner, nearly all the free fat can be extracted within twenty-four hours, all but traces of the remaining free fat can be secured within the next sixty hours, while after extraction through one hundred hours only the most minimal traces are obtainable. Tissues thus extracted will give no reactions to osmic acid, sudan III., or to scharlach R, so far as my brief experience with the latter has shown. When now this tissue is subjected to a digestion, the fat, which has to all appearances been held within the tissue in a chemical combination, insoluble in ether, is set free, can be extracted with ether, and gives the chemical tests and the reactions with the stains mentioned. This condition of resistance to extraction holds true only for ether and ligroin. When the tissue has been hardened in weak alcohol, and then later extracted with ether, less of the fat resists extraction; since this can be shown to be independent of mechanical conditions, it is apparent that the fixation has had some action in freeing the fat. To a much more distinct extent is this true of chloroform; properly manipulated, as Rosenfeld has shown, all the fat in a tissue can be extracted within a few hours with chloroform, which thus seems able to effect the separation of the components of the combination in which the fat was held.

The quantitative relations of the free and the combined fats are variable. So far as my limited experience with healthy human tissue goes, combined fat is present only in tissues of metabolic importance, and not in the connective tissues; and it is independent of the quantity of free fat. The muscular, hepatic, and renal tissues contain combined fat in notable amounts; it is present to a much less extent in the pancreas, lung, and in the alimentary tract of animals that have had no fat in the diet during several days preceding their death; bone and cartilage contain but little combined fat (a good specimen of marrow I have not yet analyzed); while from the areolar tissue, as the omentum and the subcutaneous fatty tissues, all the fat can be easily extracted with ether. The normal material at my disposal has not been sufficient to enable me to speak upon the quantitative relations in the different tissues. Four to fifteen per cent of the dried residues of muscle, liver, and kidney is fat; and of this fat from ten to forty per cent is in the form of combined fat; that is, the combined fat constitutes from less than one to four per cent of the dried residue. The higher the total fat, the lower the relative percentage of combined fat, the fluctuations occurring apparently in the free fat.

Engaged in systematic analyses of the liver and kidney in conditions of fatty degeneration, my attention was drawn to the fact that these organs when degenerated retained less of their fat through the course of an ethereal extraction than do normal organs. I further noted that they digested more easily. Inclined at first to think that the lessened amounts of combined fat were simply in proportion to and dependent upon the lessened quantity of protein in the diseased tissues, analyses directed to this point showed that no such relation existed, the reduction in combined fat was out of proportion to the reduction in protein. The obvious suggestion offered by these results was that in the course of fatty degenerations, combined fat is freed from its tissue combination. In attempting to obtain a confirmation or refutation of this suggestion in a direct analytical manner, it became obvious that this could not be done upon human tissues unless these were available in great abundance, because of the lack of control in the estimations. The amount and state of the fats in human or animal organs at different ages, under different conditions of nutrition, under different circumstances of diet, etc., are all unknown, and without such data or the possibility of a direct control no analytical determination could be possible. I have therefore tested the proposition in a direct experimental manner by means of the production of phosphorus poisoning in frogs.

The experiment was as follows: Twenty-six male frogs, rana temporania, were selected in graded sizes. They had all hibernated without food in the ranarium. They were divided into two series, in which the weights of each member of each pair corresponded quite closely. To the frogs of one series phosphorus was given, 0.0005, and repeated upon the third day. So soon as a poisoned frog died, his control-fellow was killed. The frogs died between the fourth and the tenth days. They were all dried at 45–50°, in a vacuum over pure sulphuric acid, under which conditions they dried rapidly and without decomposition. They were then prepared and extracted according to the method of Dormeyer. The two sets of material were repeatedly ground in a fine mill, which was each time washed with ether to prevent any loss. The first ethereal extraction lasted twenty-four hours, the materials were then repulverized, extracted through one hundred hours, again repulverized, and extracted a final one hundred hours. It was possible to grind the tissues to impalpable powders, and for the extractions not more than ten grammes of the powder were placed in the extraction shells. The extracts were freed of ether by evaporation, the residues taken up into hot water-free aceton, filtered, the filtrates evaporated to dryness, the residues dissolved in petroleum ether, filtered, the filtrated evaporated to dryness, the residue dissolved in ether, filtered, evaporated to dryness in appropriate vessels, dried in vacuum at 60° to constant weight and weighed. The saponification numbers were then determined for each extract. Following a complete pepsin—HCl digestion of the residues after their first ethereal extractions, they were again extracted with ether and the extracts treated just as described for the first. The results were as follows:

INITIAL WEIGHTS OF ANIMALS.

Experiment.	Control.
18.25	18.40
29.45	29.59
30.35	20.60
30.62	30.77
31.82	31.89
33.18	33.22
34.33	33.71
34.45	34.35
35.60	34.90
36.34	35.71
36.70	36.61
44.22	42.40
395.31	392.24

ANALYSES.

	Experiment.	Control.
Dried residue	85.41–21.60% of weight.	87.42–22.29% of weight.
Predigestion extract . .	7.079	7.077
Saponification number .	180.	168.
Postdigestion extract .	0.242	0.726
Saponification number .	204.7	202.8

Since the dried residue in the control material was 22.29 per cent, and in the experimental material 21.60 per cent of the original weight, and assuming that before the experiment the dried residues were alike for each group (an assumption fully warranted by the method of selection of the animals), the frogs lost during the course of the poisoning 2.9 per cent of their solid substance. This loss is explained by the increased disintegration of the tissues as the result of the disturbed metabolism. The figures emphasize anew the necessity, in work of this character, of calculating from the initial weight and not from the dried residues.

The predigestion ethereal extracts were quantitatively about the same in the two sets of material; in the postdigestion extracts the difference was marked, there was in the control material three times as much extract as in the experimental material (0.726 — 0.242). In other words, of the combined fat in the bodies of the experimental frogs two-thirds was set free during the course of the phosphorus poisoning. That the postdigestion extracts were approximately pure fat is shown by the saponification numbers.

When the sums representing the two extracts in each material are added, it is noted that in the experimental material there was less extractable substance than in the control material; the control material contained 7.803 (7.077 + 0.726), the experimental material 7.321 (7.079 + 0.242); thus there was a loss of some five hundred milligrammes of extractable substance. Does this represent a loss of fat during the experiment? Since these extracts contain non-fat it is necessary to consider the saponification numbers, which for the extract from the control material was 168, for the experimental material 180. The fat of frogs contains little tristearin, being composed largely of triolein and tripalmatine. The saponification numbers of these three fats are: tristearin 188 +, triolein 190, and tripalmatine 208. Since these saponification numbers are the lowest of any of the fats possibly concerned in such an experiment, the reduction of any saponification number below 188 must be due to the presence of nonfat in the extract under analysis. All ethereal extracts of animal tissues have saponification numbers below the numbers exhibited by their fats, and these reductions are due to nonfat soluble in ether. The more the extracts are purified by the removal of the nonfat, the higher becomes the saponification number; the first ethereal extract of frogs may present so low a number as 110. I have determined the saponification number of frog fat to be about 195, this figure having been reached after a prolonged manipulation for the removal of the nonfat, and confirmed by the calculation from the saponification number of the fatty acids isolated from frog fat. It is apparent that since the saponification number of the control fat (168) is lower than that of the experimental fat (180), the control fat must have contained more nonfat than did the experimental fat, a fact for which I know no explanation other than the suggestion that during the course of phosphorus poisoning lecothins are burned up. In order now to test the apparent reduction of the total fat in the experimental material, the approximate quantities of fat in the predigestion extracts may be determined by calculations based upon the saponification numbers. Thus calculated, the predigestion fat in the control material is determined to have been about 6.095, and in the experimental material about 6.535. The saponification numbers for the postdigestion fats were respectively 202.8 and 204.7, a little above the normal for frog fat. A saponification number above that of the main fats is due to the presence of fats or fatty acids situated lower in the series, which have a progressively higher saponification number, and especially to volatile fatty acids or their glycerides. The numbers for the volatile fatty acids could not be accurately determined for these postdigestion extracts on account of scarcity of material; in each case they were about five, a figure which while only approximate cannot have been more in error than one point above or below, that is, from 4 to 6. When this figure 5 is subtracted from the numbers 202.8 and 204.7, it is apparent that these postdigestion extracts contained practically no nonfat, since the numbers are higher than that of frog fat. The slightly higher number for the experimental fat is not sufficiently divergent to form a subject of discussion. When now to the corrected figures for the predigestion fats are added the sums for the postdigestion fats (control, 6.095 + 0.726 = 6.821; experiment, 6.535 + 0.242 = 6.777) it is seen that the fat in the control material was only some fifty milligrammes higher than the fat in the experimental material; in short, the apparent loss in fat was not an actual reduction at all, but an analytical event. In any case, fat was not formed during the experiment.

Since the above considerations bearing upon the nonfat applied only to the predigestion material, the result remains the same. For the reduction of the result to terms of figures, the corrected values for the predigestion fats will be employed. The control material contained in total fat 6.821 (6.095 + 0.726), the combined fat being 10.64 per cent of the total fat; the total fat in the experimental material was 6.777 (6.535 + 0.242), the combined fat being 3.70 per cent of the total fat. The difference between 10.64 and 3.70 (or 0.726 and 0.242) expresses the result of the experiment, demonstrating that about two-thirds of the combined fat in the bodies of frogs was freed from its metabolic combination during the course of phosphorus poisoning.

Let us now consider the result of the experiment in connection with our knowledge of the etiology and modus operandi of the general process whose results we group under the term of fatty degeneration. Under fatty degeneration we understand a complex of lesions which occurs in cells under most varied conditions of disease. The essential features consist in alterations in the structure of the cells, in disturbances in their functions, and in the appearance within the cells of fat. Concerning the disturbances in the functionation of the cells we have no exact knowledge. The primary alterations in the structure of the cell occur within the protoplasm, especially affecting the granules of the cytoplasm. The first appearances of the abnormal fat are within the protoplasm, in the form of most minute granules. Soon the nucleus shows signs of degeneration. The normal structure of the protoplasm becomes progressively disintegrated and diminished, the fat increases in quantity, and finally becomes conglomerated into droplets. In the final stage the nucleus becomes entirely degenerated and disappears, no protoplasm remains, and the entire space of the cell is filled with fat. Later the cell wall, when present, may break down, and the fat and cellular detritus lie free in the surrounding medium. Klebs first formulated an exact statement purporting to explain the phenomena; his definition, based upon some earlier physiological work, was that the essential process was the conversion of the protoplasmic protein into fat. The use of these terms must be clearly defined. By the term "origin of fat from protein" is meant the procedure defined by Voit as the formation of fat from protein by an entire reduction and subsequent reconstruction of the protein molecule, and not the formation of fat from a carbohydrate lying preformed within a complex albuminous molecule. Mucin, for example, is a glycoprotein, the micelle consisting of a protein and a carbohydrate moiety, and the carbohydrate may be easily split off; from this carbohydrate fat could of course be formed just as from any sugar, but that process would not constitute a formation of fat from protein. The identical consideration, it is necessary to insist, holds for the nucleoproteids. The nucleinic portion of these compounds may be easily split into a sugar, usually a pentose, purin bases, and phosphoric acid. Since we know that pentoses are fully assimilable, their possible conversion into fat is obvious, a consideration which seems to have been overlooked in the work designed to demonstrate the origin of sugar from protein in diabetes. Thus by the term "formation of fat from protein" when applied to nucleoproteids we should mean the formation of fat from the plain albumin component, and not from the nucleinic acid. In the strict sense, therefore, we understand the expression "formation of fat from protein" to maintain the formation of fat from a protein like casein which contain no carbohydrate, the protein being considered as disintegrated, and the carbon atoms, together with the requisite atoms of hydrogen and oxygen, being synthesized into fat, while the nitrogen, sulphur, and the superfluous oxygen and hydrogen, if such were present, are eliminated from the organism.

All the experimental work designed to demonstrate the quantitative formation of fat in connection with fatty degenerations has failed; the results, whenever experimentally correct, have been negative. The figures for the total facts in the present experiment are an additional illustration. In the meantime the process of fat transportation and deposition has been shown to be the predominating element in fatty degeneration. The plain statement is, while we have proof that fat infiltration is a strikingly prominent process in fatty degeneration, we have no evidence that fat formation occurs at all.

In a systematic survey of the process of fatty degeneration it becomes apparent that there are three possible sources of fat to be considered: the *fat in the tissue* involved at the onset of the disease; the *fat transported* from other parts of the body; and the *fat which might be formed de novo* in the affected tissue during the course of the disease. Viewing these three possible sources inversely to the order of their statement, fat may be conceived as possibly formed in the affected tissue from carbohydrates, from protein and by synthesis from fatty acids. Under the head of carbohydrates that could be considered as available would be named the circulating hemic and lymphatic sugars, glycosides, glycogen, and the carbohydrate part of compound proteins. There is evidence that in the liver, muscles, and in the blood fat is formed physiologically from sugars, but beyond that we know little. Whether in fatty degenerations fat is formed from the denominated carbohydrates in the tissue affected is a matter of which we know nothing. As regards the formation of fat from protein in the tissue affected the direct evidence is, as stated, negative. Of the synthesis of fat from fatty acids, as by the reverse action of fat splitting ferments, we know nothing in detail. We may, however, confidently expect that if fat be formed, the diastatic and lipatic processes will be found concerned in the synthesis.

Regarding the second source of fat, the work of Rosenfeld and others has taught us how widespread and predominant is fatty deposition in the tissues involved in fatty degeneration. In fact, we ought to state the problem thus: Is there, apart from the resident fat of the tissue, any other fat in the tissue in fatty degeneration than transported and deposited fat?

The fat within the tissue at the onset of the disease is present in two forms — free and combined. We do not accurately understand the normal relations and locations of the free fat of organs, whether predominately cellular or intercellular. We likewise do not know whether the form or location of this free fat be changed during the course of fatty degeneration. The experiment herein detailed has shown that in the course of fatty degeneration combined fat is set free in notable amount, a normal metabolic relation is dissociated. The relations suggest the hypothesis that this fat appears as an abnormal morphological constituent in the tissue in fatty degeneration. Upon the basis of this hypothesis the combined fat is a metabolic constituent of the protoplasm. During the course of the disease determining the fatty degeneration this combination is severed after the manner of action of ferments, the previously combined fat is set free, becomes chemically and morphologically free fat, becomes available to our technical procedures, and appears as fatty granules in the protoplasm of the cells involved. Thus viewed the initial chemical change in the cells with fatty degeneration is a sort of proteolysis, whereby the fat in combination with protoplasmic protein is set free from that combination; and the characteristic fatty appearance of the protoplasm in the early stages of fatty degeneration is due to this metabolic fat set free within the protoplasm. Following this comes fat transportation and infiltration of the affected cells, with the production of the fully-developed lesions; though why such a fat infiltration should occur, even as a passive process, we do not understand. Viewed in the light of this hypothesis, fatty degeneration is a purely disintegrating process, some of whose features resemble digestion (using the word in the broad sense of enzymic action, a better term is autolysis). How extensive may be this disintegration is illustrated by the experiment here reported, in which two-thirds of the entire fat-protein combinations of the body were disintegrated.

This theory is not new. Rindfleisch in his Lehrbuch der pathologischen Gebebslehre, 6th ed., wrote (p. 26): "Am meisten duerfte sich die Ansiche empfehlen, dass wir es mit Erscheinungen zu thun haben, denjenigen entgegengesetzt, welche die Zellenbildung begleiten. Wie wir aus der Zusammensetzung des Dotters wissen, besteht das Bildungsmaterial der Zellen aus Eiweisskoerper, welche reichlich mit Fett gemischt sind. Wir wissen ferner aus der chemischen Analyse der Muskelfasern, dass dieselben eine nicht unbedeutende Masse unsichebaren Fettes enthalten, so dass wir Grund haben, eine amalgamartige Verbindung von Fett und Eiweisskoerpern in den Zellen anzunehmen. Die fettige Degeneration ist eine 'Wiederscheidung' dieses Amelgams, bei welcher das Fett frei wird, und in groesseren Troepfchen im Protoplasma erscheint."

EXPERIMENTAL FAT NECROSIS.[1]

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Langerhans[2] made the first actual attempt to learn the cause of the changes of fat necrosis in 1890. Before that time Balser[3] (1882), who first called attention to the condition, and Chiari[4] (1883) had attempted to reach conclusions by study of the histology of the lesions. The former decided that overgrowth of fat tissue was at the bottom of the trouble, thinking that the new fat cells which he described as growing about the periphery shut off the blood supply of the centre of the focus. Chiari took an exactly opposite view. Observing fat necrosis in the bodies of marantic patients, he considered that lack of nutrition during cachexia was the cause. From his histologic studies he concluded that the process was not at all different from retrogressive changes seen in other organs, and there designated as simple necrosis and fatty degeneration.

Langerhans' first studies were of a histologic and microchemic nature. He observed that the necrotic masses did not float like masses of stearin, but sank quickly to the bottom. On teasing out they were seen to consist of characteristic needles of fatty acids, a few small oil droplets, and many flakes of varying size, the last being responsible for the high specific gravity. Further evidence of the change from the normal fat that had previously occupied the affected area was furnished by staining with osmic acid, which merely yielded a dirty, grayish-brown coloration. Addition of concentrated sulphuric acid to sections from which the fat had been removed with alcohol and ether caused complete solution of the heavy flakes. If this treatment was followed by the addition of water and the specimen allowed to stand until the following day, numerous characteristic crystals of calcium sulphate settled out. From these observations he concluded that since both calcium and fatty acids existed in the necrotic areas they were probably combined. Further evidence was furnished by the insolubility of the calcium salts in hydrochloric acid, which showed that they were not carbonates or phosphates, and the insolubility of the fatty acids in boiling ether, which showed that they were not free. At the time Langerhans wrote the knowledge of the history of fat in metabolism was less advanced than now, and he sought support for his assumption of the formation of calcium soaps in the observations on this process, as it occurs in adipocere. With this exception the chief illustration of such a process was the description of retrogressive metamorphosis in lipomas by Virchow,[1] who said that in these tumors the fat is occasionally saponified, combines with calcium and magnesium, and together with earthy phosphates forms a crumbling, mortar-like mass. By studying fresh specimens, Langerhans sometimes found foci in the middle of the fat lobule, consisting of fat cells full of fatty acids without calcium salts, showing that the splitting occurred first and the union with calcium later. His conception of the process after these studies he states as follows: "To collect these observations briefly, it develops that the multiple necroses of fat tissue begin with decomposition of the neutral fat contained within the cells; the fluid constituents are eliminated and the solid fatty acids remain behind. The latter combine with calcium to form calcium fatty-acid salts. The entire lobule, or several neighboring lobules, form a dead mass, which has the destroyed separated from the living tissue through a demarcating inflammation." The part played by the fat cell itself in the necrosis was puzzling. He found that the nucleus did not lose its affinity for stains until after decomposition of the contained fats. He says:

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[1] A portion of this paper is published in the Decennial Publications of the University of Chicago, Vol. 10, 1903. Presented with specimens before the Chicago Pathological Society, Jan. 12, 1903. Received for publication Jan. 7, 1903.

[2] Virchow's Archive, cxxii, 1890, 252.

[3] Virchow's Archive, xc, 1882, 520.

[4] Prager Med. Woch, 1883, No. 30, 285, No. 31, 299.

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[1] Krankhaften Geschwülste, Bd. I., s. 393.

"Although there is no demonstration of a primary injury to the cell, I cannot hazard the conclusion that the physiological function of the cell is intact at the beginning of the decomposition of the fat, because we know so little of the rôle of the cells in the building and taking up of fat. It would, perhaps, be simplest to assume that in metabolism somewhere within or about the cell a harmful substance appeared and accomplished the decomposition of the fat droplets, but, unfortunately, every observation fails to confirm this."

The frequent association of fat necrosis with lesions of the pancreas, the frequent localization of the process to the fat about the pancreas, and the similarity of the fat-splitting that he had described with that of pancreatic digestion, led Langerhans[1] to study the relation of this organ to the disease experimentally, and in 1891 he described his methods and results. He made an extract of the pancreas of freshly killed rabbits by rubbing the gland in a mortar with splintered glass, under aseptic precautions. This was injected after filtration into twelve animals, nine rabbits and three dogs, and in one rabbit a single spot of typical fat necrosis appeared in the peri-renal fat. From this one success he believed that the pancreatic juice was the cause of the fat necrosis, but of course he could not obtain any clear conception of the process.

Since that time many sorts of experiments have been tried in the study of fat necrosis, most of which have simply added a new method by which pancreatic juice can be made to enter the peri-pancreatic fat, or the subcutaneous fat (Williams), and the abdominal cavity, when, all are agreed, fat necrosis frequently but not invariably results. Experiments in which extracts or dried preparations of the pancreas have been used, rather than the secretions of the living gland, have been made by Jung and by Dettmer.

Jung[2] placed a gelatin capsule containing "trypsin" in the abdominal cavity of one rabbit, and pieces of dog's pancreas in three others. All showed fat necrosis, which he thought was due to the fat-emulsionizing and fat-splitting ferment, but the greater part to the proteid-splitting enzyme. The experiment upon which Jung based his idea of the importance of trypsin was the one mentioned first, in which a preparation of pancreas in a gelatin capsule was placed in contact with the omentum. As this preparation, which he calls "trypsin," seems to have been merely dried and pulverized pancreatic tissue, his assumption of a pure tryptic action is quite unwarranted, since the other ferments are not excluded.

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[1] Festschrift zur Feier des 71 Geburtstages Virchow's.

[2] Dissertation, Göttingen, 1895.

Dettmer[1] first tried the effect of causing pancreatic juice to enter the abdominal cavity, which was accomplished variously by ligating the pancreatic duct, or the vessels, or both; by placing pancreas tissue in the abdominal cavity, and by injecting an extract of fresh pancreas. As these caused fat necrosis, he endeavored to find what ferment was responsible, to which end he injected trypsin into still other animals. As trypsin alone did not cause fat necrosis he concluded that the fat-splitting ferment must be responsible. The trypsin used by Dettmer was quite a different article from that used by Jung, and he describes it as follows: "The trypsin used by us (from the firm of Merck in Darmstadt) was studied thoroughly after it was softened in water; it showed no trace of organic structure, but had a more crystalline appearance; it was designated by the firm as the proteid-splitting substance, in contrast to another preparation, called pancreatin, which contains all active elements of the pancreas. A test of our preparation for a saccharificant action gave a negative result, likewise the test for fat-emulsionizing property. Our preparation was therefore pure trypsin. We obtained with our pure preparation only hemorrhage, no fat necrosis in the Balser-Langerhans sense." It will be observed that no test for lipolytic activity was made. The experiments performed by Dettmer were as follows:

Experiment 1: Injected into the peritoneal cavity of a cat "a large quantity of a watery solution of trypsin." Thirteen days later, the cat having remained well, the abdomen was opened aseptically and nothing abnormal found.

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[1] Dissertation, Göttingen, 1895.

About one gram of powdered trypsin was then sprinkled upon the omentum and intestines of this cat. Experiment 3: The animal died in less than twenty-four hours, and autopsy showed great hyperemia and many hemorrhages in the omentum, but the fat itself "showed no traces of alteration." There was no evidence of peritonitis. Experiment 2 was a duplicate of Experiment 1, with likewise a totally negative result. This animal then had about one-half gram of trypsin placed in the abdomen, after which the wound was closed. Experiment 4: Death occurred seven days later. Autopsy showed no peritonitis, the fat was entirely unaltered, but there were a few small hemorrhages in the omentum. The parts of the intestine that had come in contact with the trypsin were superficially eroded. No histologic examination is mentioned. Dettmer's conclusions are as follows: "These experiments show beyond question that the proteid-splitting ferment, the trypsin, is not able to produce fat necrosis. But as fresh pancreatic juice is always able to cause fat necrosis in living fat tissue, there remains nothing more possible except that this action is due to the third ferment contained in pancreatic juice, the fat ferment."

Except for the series of experiments just mentioned, the numerous experiments performed by many different investigators have, for the most part, added nothing to the important matter of how the pancreatic juice causes this peculiar form of necrosis, and what constituent of the juice is the active one. Their results have shown that fat necrosis can be obtained in the following ways: By injecting aqueous extracts of the fresh gland; by transplanting pieces of pancreas; by ligating the pancreatic duct, with or without laceration of the organ, to facilitate escape of the pancreatic juice; by ligating the veins leading from the pancreas, with or without injury to the gland or ligation of its duct; by injection of necrotizing or infectious substances into the gland itself or into its duct; by cutting the duct or bisecting the organ, so that its secretion escapes into the surrounding tissues; by binding between glass rods, and other forms of trauma. All of these methods have close relation to the conditions of trauma, hemorrhage, and infection which ordinarily are the cause of fat necrosis as seen in man. These experiments, as well as clinical observations, taken all together leave no doubt that pancreatic juice, and it alone, is the cause of fat necrosis, bacteria having been excluded by many negative cultural experiments.[1] Further than this they tell little.

In contrast to them is the work of Flexner,[2] in which was attempted to prove definitely the relation of pancreatic lipase to fat necrosis, which relation had, since Langerhans's one successful experiment, generally been assumed to be a positive one. Flexner's studies were directed to prove the presence of lipase in the areas of necrosis in both human and experimental cases, and its absence in the normal fat tissue. His method is described as follows: A neutral fat was prepared from fresh butter by dissolving it in ether, adding a few drops of NaOH solution, and washing out with water. The separated ethereal solution of neutral butter was evaporated over a water bath, and the tests were made at once. For this purpose were employed (1) a piece of the pancreas itself, (2) one or more focal necroses, and (3) a piece of adipose tissue equal in size to the pieces of pancreas or necroses, this piece being taken from a distance from the necroses. The pieces of tissue were placed in ninety per cent alcohol for from one-half to two hours, pressed in filter paper, reimmersed in the alcohol for a short period, again pressed and allowed to become air-dry. They were then cut into morsels, which were incorporated with the neutral fat in watch glasses which were then covered and placed in the thermostat at body temperature. The fatty acids liberated were demonstrated by the odor and by tincture of litmus, the reaction being sometimes obtained, it is stated, within six minutes. Fat necrosis was produced in cats and dogs by various methods leading to escape of juice from the gland, most satisfactory of which was combined ligation of the veins and laceration of the gland. In one case of fat necrosis Flexner was able to obtain a positive reaction in ten minutes from the necrotic tissue and from the pancreas, but not from the normal fat tissue. In animals killed on the second, third, and fourth days all gave evidence of fat-splitting enzyme in the necroses, but not in the normal fat. To test the time at which the lipase (steapsin) disappeared, pieces of necrotic tissue were excised aseptically from cats on the third day after the operation on the pancreas, and the animals allowed to live until the sixth and eighth days. The conclusions drawn from the experiment were: "(1) In peritoneal fat necrosis the fat-splitting ferment is demonstrable at certain stages of the pathological process; (2) it is present in greatest amount in the early stages and may disappear in the later ones when healing is well advanced; (3) although it cannot be affirmed that steapsin is the direct cause of the necrosis of tissues, such an assumption is rendered highly probable by its constant occurrence in the diseased areas, its absence from the healthy fat, and the nature of the pathological changes; (4) the escape of the pancreatic secretion into the peri- and parapancreatic tissues is the origin of the necroses, and this escape is facilitated chiefly by lesions of the pancreas itself, but also by disturbances in its circulation." Flexner also adds: "That necrosis of fat cells may result from other causes is certainly not excluded by these findings. The genesis of similar lesions found in the marrow of the bones by Ponfick, in the subcutaneous fat (Chiari) and pericardial fat (Balser, Chiari) is not immediately apparent. That micro-organisms may, in these cases, play a part is, in my opinion, very probable indeed."[1]

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[1] The chief exception to this statement is furnished by the remarkable case reported by P. Wulff (Berl. klin. Wochenschr., 1902, xxxix, 734). It occurred in a man who had been drinking heavily, and who presented symptoms indicating perityphlitis, for which he was operated. Extensive necrosis of the subcutaneous fat of the right lower quadrant of the abdomen was found, communicating with a large retro-cæcal cavity into which extensive hemorrhage occurred and caused death some time after the operation. About this cavity there was found at autopsy massive fat necrosis, and many minute foci were found in the omentum, mesentery, pericardium, peripancreatic and retroperitoneal fat tissue. The pancreas was perfectly normal, macro- and microscopically. This case is quite unique in the literature, and unless something was overlooked at the autopsy it is difficult of explanation. The conclusion of Wulff that the pancreatic lesions are often secondary to fat necrosis, which is a disease *sui generis*, seems hardly warranted on the basis of a single case.

[2] Journal of Experimental Medicine, 1897, ii, 413.

This valuable work of Flexner's, filling out the results of Langerhans and Dettmer, seemed to leave little doubt that fat necrosis is simply a matter of escape of pancreatic juice into the fat tissue about the gland and into the peritoneum, and the lipase (steapsin) acting upon the fat causes its splitting, which leads to the peculiar and characteristic foci. To be sure Flexner appreciated the incompleteness of the demonstration and the many unsolved questions, but this has been generally overlooked, and at the present time the teaching is usually as above stated.

The evidence upon which is based the belief in the primary importance of lipase in the pathogenesis of fat necrosis, human and experimental, is as follows: (1) The microscopical evidence of fat splitting, consisting in the demonstration of masses of crystals of fatty acids in the areas of necrosis, together with the disappearance of the fat from these areas; (2) the association of fat necrosis with the occurrence of free pancreatic juice, the known presence of lipase in this fluid, and the inability to produce fat necrosis with the tryptic ferment (Dettmer; the experiments of Jung seem to be of little or no value); (3) the demonstration by Flexner and by Opie of a fat-splitting ferment in the areas of necrosis, while it could not be demonstrated in the normal fat tissue.

The course of events is believed to be about as follows: (1) An injury to the pancreas by infection, trauma, occlusion of ducts (Opie), or what not; (2) escape of more or less normal pancreatic juice either free into the tissue or into the lymph stream; (3) dissemination of the fluid, in a manner not always easy to explain; (4) action of the lipase upon the fat cells, leading to splitting of their contained fat into glycerin and fatty acid; (5) glycerin diffuses, leaving behind the solid fatty acids; (6) necrosis of the cells; (7)

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[1] A full corroboration is furnished by E. L. Opie (Contributions of the pupils of William H. Welch, Baltimore, 1900, 859), who, using Flexner's method, obtained reactions with foci remote from the pancreas, namely, the pericardial and subcutaneous fat, in animals in which widespread dissemination of the lesions had been produced.

eventually the fatty acids combine with calcium salts and are precipitated, while a new growth of connective tissue encapsulates the area and diminishes its size.

All this is simple and clear cut, but unfortunately far from proved. This is particularly so when the part supposedly played by lipase is considered in the light of recent developments in the study of fat and fat metabolism. J. H. Kastle and A. S. Loevenhart[1] have demonstrated not only that lipase is present in all of the tissues of the body in which there is any utilization or storage of fat, but also that lipase acts reversibly upon fat and its components, building up fat from the fatty acids and alcohol as well as splitting fat into these substances. This reversibility of enzymes had previously been shown for maltase by A. C. Hill; and for the ferment that synthesizes benzoic acid and glycocoll into hippuric acid, by Schmiedeberg, who, however, did not appreciate the significance of the reactions that he had observed. The work of Kastle and Loevenhart may be briefly summarized as follows:[2] Lipase will cause solutions of ethyl alcohol and butyric acid to unite to form the ester, ethyl butyrate. On the other hand, in solutions of ethyl butyrate, lipase causes a splitting. In either case the end result is the same, namely, a mixture of fat, fatty acid, and alcohol; in other words, lipase simply acts to establish an equilibrium between these substances, and the effect of the enzyme is merely to hasten this equilibrium which would be attained more slowly without its aid. Subsequently it was found that all the tissues in the body that were tested for lipase showed its presence, most notably in the liver, active mammary gland, blood, lymph, and intestinal mucosa, as well as, of course, the pancreas. Of particular interest is the fact that the liver is about two times more active, weight for weight, than the pancreas of the hog, and of course in its total bulk many times more. Subcutaneous fat was found to be both lipolytic and lipogenetic, as also are the pericardial and perinephritic fat, showing the presence of lipase in adipose tissue.[1]

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[1] Chemical News, 1901, lxxxiii, Nos. 2150 to 2155; and also,
American Chemical Journal, 1900, xxiv, 491.

[2] For a more complete discussion of fat metabolism see "On the Relation of Lipase, to Fat Metabolism — Lipogenesis," by A. S. Loevenhart, Amer. Jour. of Physiology, 1902, vi, 331; also "Reversibility of Enzymes and its Application to Physiological and Pathological Processes," H. Gideon Wells, Journal of the American Medical Association, 1902, January 25.

The history of fat in the body may now be considered to be as follows: The lipase of the stomach does not act because of the presence of hydrochloric acid. In the intestines lipolysis occurs, with production of a mixture of fat, fatty acid, and alcohol, usually glycerin; but as the fatty acid and glycerin are diffusible, while the fat is not, they are separated from the fat by absorption into the wall of the intestine. Hence an equilibrium is not reached in the intestine, so the splitting continues until practically all the fat has been decomposed and the products absorbed. When this mixture of fatty acid and glycerin first enters the epithelial cells lining the intestines there is no equilibrium, for there is no fat absorbed with them as such. Therefore the lipase, which Kastle and Loevenhart showed was present in these cells, sets about to establish equilibrium by combining them. As a result we have in the cell a mixture of fat, fatty acid, and glycerin, which will attain equilibrium only when new additions of the two last substances cease to enter the cell. Now another factor also comes in, for on the other side of the cell is the tissue fluid containing relatively little fatty acid and glycerin. Into this the diffusible contents of the cell will tend to pass to establish an osmotic equilibrium, which is quite independent of the chemical equilibrium. This abstraction of part of the cell contents tends to again overthrow chemical equilibrium, there now being an excess of fat in the cell. Of course the lipase will, under this condition, reverse its action and split the fat it has just built into fatty acid and glycerin. It is evident that these processes are all going on together, and that as the composition of the contents of the intestines and of the blood vessels varies, the direction of the enzyme action will also vary. In the blood serum, and also in the lymphatic fluid, there is more lipase which will unite part of the fatty acid and glycerin, and by removing them from the fluid about the cells favor osmotic diffusion from the intestinal epithelium, thus facilitating absorption. Quite similar must be the process that takes place in the tissue cells throughout the body. In the blood plasma bathing them is a mixture of fat and its constituents, probably nearly in equilibrium, since lipase accompanies them. If the diffusible substances enter a cell containing lipase, *e.g.*, a liver cell, the processes of building and splitting will be quite the same as in the intestinal epithelium. The only difference is that here oxidization or other chemical processes may remove the fatty acid and glycerin from the cell.

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[1] It will be noted that these results do not agree with those of Flexner, which seemed to show the presence of lipase in foci of fat necrosis, but not in the normal fat tissue. There are several possible explanations for this discrepancy. One is that the amount of lipase present in the small pieces of normal tissue used was so small that it did not manifest itself in the relatively coarse method used; that it did appear in the foci of necrosis may be because of the disorganization of the tissue permitting much more effective extraction of the lipase than could be obtained in the highly impermeable normal fat tissue. Again, it is highly possible that the enzyme that splits ethyl butyrate is not the same as the one splitting tributyrine, which Flexner used. And of course the pancreatic lipase from the extravasated juice would have entered the necrotic areas more than the normal tissue, whether the cause of the necrosis or not.

With this explanation of the physiological processes of fat metabolism in mind, a reconsideration of the subject of fat necrosis leaves one in considerable confusion. (1) Since lipase is a normal constituent of fat tissue, how can it be that the presence of lipase of pancreatic juice is responsible for the changes of fat necrosis? (2) The splitting of fat occurring normally and constantly in the fat cells, is it possible that the products of this decomposition or the lipase itself can cause the death of the fat cells? (3) The presence of masses of free fatty acids in the fat tissue does not at all agree with the idea of reversible action of lipase. If the action of lipase is to cause a balance between the fats and their acids and alcohol, the presence of added lipase in the fat tissue should have little effect; the only one conceivable would be an increased accumulation of fat in no wise different from that due to increased ingestion of fats. (4) Ferments are not diffusible, yet one not infrequently finds the lesion deep in the fat tissue when the lipase has been simply introduced into the peritoneal cavity. Fat necrosis not infrequently occurs as a widespread process, invading not only the entire peritoneal fat but also the subcutaneous, pericardial, and subpleural fat. It is difficult to explain this distribution and localization of a ferment. The focal character of the lesion is equally perplexing.

All these questions and facts leave much doubt as to the genesis of fat necrosis through action of lipase, while they offer no substitute explanation. The facts of which we are sure, and upon which must be based the study of the problem, are the following: (1) An actual necrosis of fat tissue occurs which is sharply circumscribed; (2) within the necrotic area splitting of the fats takes place, leading to the presence of free fatty acid crystals in large masses; (3) these lesions are present in man only associated with escape of pancreatic juice from its normal channels, and has been produced in animals always by similar processes; (4) the etiological factor may be distributed to points remote from its original site.

The questions to be answered are: (1) Is a ferment responsible for fat necrosis, or can it be produced by agents that are not ferments? (2) If a ferment, is it lipase or one of the other ferments of the pancreatic juice or of the tissues? (3) If lipase, is it the lipase of the pancreatic juice or of the fat tissue itself that is acting? (4) Is the necrosis of the tissue primary and the splitting of the fat secondary, or, as seems to be commonly accepted, is the splitting of the fat the first step and the necrosis a result of this lipolysis? (5) How is the peculiar dissemination of fat necrosis brought about, and why is the process focal?

IS A FERMENT CONCERNED IN FAT NECROSIS?—It is quite remarkable that in the experiments so far performed no attempt has been made to determine this point by the usual method for detecting ferment action, that is, the susceptibility of ferments to heat. Probably this is because nearly all experiments so far have been by causing an injured or transplanted pancreas to yield its secretion intra-vitam, under which circumstances study of the acting fluid was not easy.

To determine the actual presence and agency of a ferment it was necessary to secure an extract that could be manipulated outside of the body, and that would produce fat necrosis with a considerable degree of certainty. In view of Langerhans' results, but a single focus in twelve experiments, the simple preparation made by triturating fresh pancreas with water did not offer a good prospect, but as it was the simplest it was tried first. The results were surprising, in that the extract of fresh hog's pancreas never failed to cause a decided fat necrosis in cats, dogs, and rabbits. Probably Langerhans' poor success was due to the much slighter activity of the pancreas of the rabbit, which he used, as compared with that of the hog. Loevenhart found that hog's pancreas gives a much more actively lipolytic extract than does that of the dog, and it is quite probable that the herbivora would have a still weaker pancreatic juice. The extract used was made as follows: Fifty grams of hog's pancreas, removed about three hours before the experiment, was washed quickly in sterile water, then in ninety-five per cent alcohol, and again in sterile water, to remove as many as possible of the bacteria on the surface of the gland, necessarily present from the handling at the slaughter-house. It was then minced in a sterilized meat chopper, collected in sterile dishes, and triturated with sterile quartz sand in one hundred cubic centimeters of the solution to be used. In one series the fluid was one per cent sodium carbonate, and in another four-tenths per cent acetic acid. The gland had of itself a slight initial acidity to phenol-phthalein. After standing a short time to permit the sand to settle out, the fluid was strained through gauze, which permitted a considerable amount of pulp to pass into the filtrate. From ten to fifty cubic centimeters of this fluid was then injected into the abdominal cavity of cats, dogs, and rabbits, after the various indicated manipulations had been performed.

Protocol 2. *Dog, received intraperitoneal injection of an alkaline solution of fresh hog's pancreas. Killed after five days, and fat necrosis found.*

II. — 11 — '02. Injected intraperitoneally 50 cc. of emulsion of fresh hog's pancreas in 1 per cent Na_2CO_3.

II. — 16 — '02. Appears perfectly well, and has been so ever since the first day after the injection. Killed with chloroform. Autopsy: Omentum lies over the upper half of the abdominal cavity, adherent by firm hemorrhagic fibrinous adhesions to the parietal wall and to the intestines. A mass of intestines is adherent beneath the omentum, and in the coils are a few small pockets of pus. Scattered about in the omental fat are many irregular areas, pinhead size and larger, appearing somewhat more yellow than the fibrin masses. There are a few such areas in the mesentery. The areas are not all near the point of greatest inflammation, but some are in fat that appears otherwise normal. Pancreas and other viscera appear normal. Cultures from the abdominal fluid yielded a bacillus in morphology like B. coli communis. Histologically the areas described are seen to be typical foci of fat necrosis, with considerable leucocytic demarcation.

Protocol 51. *Cat, received intraperitoneal injection of an alkaline solution of fresh hog's pancreas. Death after about twelve hours; typical fat necrosis.*

IV. — 22 — '02, 11.30 A.M. Injected intraperitoneally, under chloroform anæsthesia, 30 cc. of emulsion of fresh hog's pancreas in 1 per cent Na_2CO_3.

IV. — 23 — '02, 8 A.M. Found cold and stiff. Probably had not survived the injection more than twelve hours. Autopsy showed an extensive fibrinous deposit over all the peritoneal surfaces, with a considerable quantity of turbid, pinkish fluid. In the omental fat were several areas that seemed to be typical fat necrosis. Pancreas and other viscera showed no change. Cultures from the fluid yielded the staphylococcus pyogenes aureus, and an undetermined bacillus. Histologically there were found in the omentum, in addition to typical minute foci of fat necrosis, areas of more general necrosis affecting all of one side of the omentum, the other side being unaffected, a sharp line of demarcation between the necrotic and the unaffected tissue being present. There were some places where the necrosis extended entirely through the omentum.

Protocol 56. *Cat, injected with alkaline emulsion of pancreas. Death after eighteen hours; result positive.*

IV. — 29 — '02, 1.45 P.M. Ten cc. of a 10 per cent solution of Na_2CO_3 containing emulsion of fresh hog's pancreas injected intra-peritoneally.

IV. — 30 — '02, 9 A.M. Found dead, but body still warm. About one ounce of turbid fluid found in the peritoneal cavity, the surfaces being cloudy and hyperæmic. A few foci of necrosis seen in the pro-peritoneal fat, retroperitoneally, and in the mesentery; more in the omentum, but nowhere very abundant. The largest are the size of a pinhead. There are also stripes of fat necrosis in the omentum extending along the fat trabeculæ. A few foci in the pericardial fat in the lower part, but none in the fat above the heart. Pancreas and other organs show no changes. Cultures: An agar slant developed a profuse growth, but was not worked out. Histologically typical fat necrosis in foci and *en masse* as in 51.

Protocol 50. *Dog, injected with acid extract of pancreas, death in about twelve hours; result positive.*

IV. — 22 — '02, 1.30 P.M. Injected with 50 cc. of an acid extract of fresh hog's pancreas, made by triturating the gland substance with 0.4 per cent acetic acid; after straining through cheese-cloth the fluid was allowed to stand 1½ hours at room temperature, to permit the acid to have whatever effect it might upon the enzymes present.

IV. — 23 — '02, 8 A.M. Found dead and cold; could not have survived the injection more than twelve hours. Autopsy: Peritoneal cavity contains turbid, fibrin-flecked fluid. Omentum slightly adherent over intestines and studded with clear white spots, from pin-point size to confluent areas one or more centimeters in diameter. The mass of fat attached to the anterior abdominal walls is studded with similar spots, without evidence of inflammation. Smaller numbers were also present in the retro-peritoneal, mesenteric, and pericardial fat tissue. Cultures all gave growth of an organism resembling, on agar slants, the growth of B. mucosus capsulatus, but it was not followed out. Histological examination shows typical fat necrosis.

Protocol 53. *Cat, received acid extract of pancreas. Death in about twelve hours; result positive.*

IV. — 22 — '02, 2.30 P.M. Injected 20 cc. of an emulsion of fresh hog's pancreas in 0.2 per cent acetic acid, after it had stood for about 2½ hours.

IV. — 23 — '02, 8 A.M. Found dead and cold; must have died not much later than twelve hours after injection. Autopsy: Peritoneal cavity contained much turbid fluid and fibrin. The animal was emaciated, and the fat was small in amount, but in the omentum were occasional fairly typical foci, and a few others in the mesentery, but none found elsewhere. Histologically the fat necrosis was found to be typical, but more extensive than appeared to the naked eye, the entire surface of the omentum in considerable areas showing a necrosis for a depth of one or two cells.

Protocol 60. *Cat, received injection of pancreas emulsion in acid. Death after eighteen hours; result positive.*

IV. — 29 — '02, 4 P.M. Injected into peritoneal cavity 20 cc. of an emulsion of fresh hog's pancreas in 0.4 per cent acetic acid, which had stood two hours.

IV. — 30 — '02, 10 A.M. Found dying, and chloroformed. Typical fat necrosis, moderate in abundance, distributed generally throughout the omentum, mesentery, and retro-peritoneal fat, the largest areas being not larger than a pinhead. There were also several typical areas in the pericardial fat, but none above the heart. The contents of the peritoneal cavity were slightly acid to phenol phthalein. Cultures showed abundant growth of unidentified organisms. Histological examination shows most extensive fat necrosis, often passing entirely through the omentum in large areas, and notable for the absence of leucocytic invasion.

Protocol 52. *Rabbit, injected with pancreas emulsion, alkaline, that had been boiled. Inflammation, but no fat necrosis.*

IV. — 22 — '02, 3 P.M. Injected with 30 cc. of the same emulsion as No. 51, that is, in 1 per cent Na_2CO_3, after it had been heated to boiling in the water bath for 20 minutes.

IV. — 23 — '02, 10 A.M. Seems well; killed. Abdominal cavity contains a considerable amount of fibrinous exudate, not greatly different in amount and nature from that seen in the animals injected with unboiled extract, and no traces of fat necrosis. Cultures remained sterile. Histological examination of the omentum, which was preserved entire and examined throughout, showed no fat necrosis, merely acute inflammation.

Protocol 58. *Cat, injected with alkaline pancreas emulsion that had been heated to boiling. Killed after three days; result negative.*

IV. — 29 — '02. Injected 20 cc. of pancreas emulsion in 1 per cent Na_2CO_3 that had been heated 10 minutes at 100° C.

V. — 2 — '02. Has been quite well. Killed by chloroform. Peritoneal cavity shows a few threads of fibrin, otherwise no evidence of inflammation, and no signs of fat necrosis. Microscopic examination showed a slight leucocytic infiltration, but no fat necrosis.

Protocol 61. *Cat, received alkaline pancreas emulsion that been heated to boiling. Killed at the end of three days. Result negative.* A duplicate of No. 58.

It will be seen that not in a single case did the unboiled hog's pancreas, whether in alkaline or acid solution, fail to produce a distinct fat necrosis, while the injections of similar preparations that had been boiled failed to cause any such process, macro- or microscopical. It is thus certain that in the fresh hog's pancreas there is some substance that is destroyed by boiling, and without which the pancreatic extract is unable to cause fat necrosis. The chief criticism of these experiments is that bacteria were not excluded — there were culturable organisms in the peritoneum of all the animals with fat necrosis, while cultures from the animals injected with boiled extract were sterile, showing that the bacteria were present in the pancreatic extract as prepared. The treatment necessary to secure an active emulsion of pancreas renders it difficult, if not impossible, to maintain sterility, but fortunately it was found not to be necessary to obtain pancreas extracts in this way to secure sterility. It was found that the ordinary commercial "pancreatin" prepared by various firms was invariably effective, even when the preparation used was several months old.[1]

There was never a failure to produce fat necrosis by injecting intra-abdominally into a cat or dog as much as ten cubic centimeters of a five per cent suspension of this preparation, and often the process was very extensive, even more so than any described from the operative methods used by various experimenters. It made no difference whether the solution was alkaline, acid, or neutral, as with fresh pancreas. Experiments made to ascertain the minimum amount of pancreatin that would cause fat necrosis showed that amounts as small as 0.05 gm. of commercial pancreatin might sometimes produce a few foci, and again sometimes fail entirely.

Because of its simplicity and effectiveness this method can be recommended for teaching purposes, as it gives a ready method for demonstration of the appearances of fresh fat necrosis as seen by the surgeon. The frequency with which surgeons mistake this condition for miliary tuberculosis leads one, in fact, to urge that this demonstration be frequently made.

Pancreatin seems to be free from pathogenic bacteria, probably because of its dry condition; at least cultures from animals with fat necrosis produced by this preparation were quite generally sterile. This, therefore, excludes bacteria as a causative agent in these experiments, an exclusion not absolutely essential, as many investigations have already shown the non-bacterial nature of fat necrosis. Marx[2] reviews the literature of this subject, and finds that in human beings the results have been variable, colon bacilli being often found and sometimes cocci, especially when accompanying marked pancreatitis. The very variability of these results is in favor of the accidental nature of the presence of bacteria. Further, in acute peritoneal infections of other than pancreatic origin fat necrosis is not found. In experimental fat necrosis Opie found that pieces of necrotic fat were sterile.

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[1] Preparations put out by the following firms were tried, and all gave positive results: Armour & Co., Truax, Greene & Co., Parke, Davis & Co., Fairchild Bros. & Foster, and Merck (not the scale preparation). In these, as in all subsequent experiments, unless otherwise specified, the preparation of Armour & Co. was used, not because of any particular activity, but because it was first used and it seemed desirable to use the same preparation throughout to make the results comparable.

[2] Virchow's Archives, 1901, clxv, 290.

As with the fresh pancreatic extract, boiling was found to prevent the production of fat necrosis, without fail, no matter what the reaction of the fluid. It now remained to ascertain the temperature at which this property was destroyed.

THERMAL DESTRUCTION POINT. — A series of experiments was performed, in which one gram pancreatin in twenty cubic centimeters distilled water was kept for a period of five minutes at temperatures within a narrow range; then, after cooling, ten cubic centimeters containing about one half gram was injected intraperitoneally. Abstracts of the protocols follow:

Protocol 79. Medium-sized dog injected intraperitoneally with 10 cc. of emulsion of distilled water and Armour's pancreatin, 0.5 gm., that had been heated five minutes between 55° and 60° C. After three days it was killed, and abundant and typical foci were found in the omentum, and in the fold of the anterior peritoneum.

Protocol 82. Cat received intraperitoneally 0.5 gm. pancreatin in water that had been heated to 70°, and then slowly cooled to 60° during fifteen minutes. Killed after two days. At one point in the omentum were found a few minute white points grouped together, which microscopic examination showed to be areas of fat necrosis, with considerable infiltration with leucocytes.

Protocol 81. Dog received emulsion of pancreatin that had been heated for five minutes at from 68° to 70°. Killed after two days, and in the omentum were perhaps a dozen minute pin-point sized spots which were seen microscopically to be foci of fat necrosis, marked by intense invasion of leucocytes.

Protocol 87. Twenty cc. of water containing about 1 gm. of pancreatin was poured through an abdominal wound in a cat, upon which had been performed a laporatomy for the purpose of removing part of the omentum in connection with another experiment. The mixture had been heated for five minutes at from 65° to 71°. Killed after two days, and about the site of the ligature on the omentum the fat was found streaked with white, which in some places forms considerable areas of changed fat. More remotely in the omentum and in the mesentery were a very few minute white spots. All these white portions of the fat were found under the microscope to be necrosed fat.

Protocol 107. A cat received 10 cc. of emulsion of pancreatin that had been heated to from 71° to 74° for five minutes. Killed after two days and no traces of fat necrosis could be found.

Protocol 91. Small dog received an injection of 10 cc. of pancreatic emulsion that had been heated for five minutes at from 75° to 79°. Killed after two days and no fat necrosis could be found.

Protocol 94. Through a laparotomy wound in a small dog was poured 15 cc. of an emulsion containing 0.5 gm. pancreatin, which had been heated at from 80° to 81° four minutes. Killed after two days and no fat necrosis could be found.

As will be seen at temperatures not over 60° the pancreatin is still quite active, but less so than when unheated. Two experiments at temperatures reaching 70° C., but staying for the greater part of the time a few degrees lower, gave a few foci that were characterized by early invasion of leucocytes and healing. A third preparation of the same temperature used in an animal that had suffered an injury to the omentum immediately before, caused a much more marked necrosis, chiefly about the site of the injury where the action of the agent seemed to have been favored. Temperature above 71° C. invariably destroyed the property of the pancreatin to cause fat necrosis. Probably the destruction point of the active agent is not a fixed one, but will vary within slight limits in different preparations and in solutions of different reactions.

The agent contained in extracts of fresh hog's pancreas and in dry commercial pancreatins is, therefore, destroyed by heat; in the case of the latter the maximum temperature that can be withstood for five minutes in an aqueous solution is not above 71° C. This observation seems to indicate that we have here to deal with a ferment, almost certainly, one is tempted to say, for the best known ferments suffer loss of activity at just about such temperatures. The close agreement of the experiments pointing to a reduction of activity at temperatures from 65° C. to 71° C., and the entire loss of power above this point seemed to offer a hopeful field for study to ascertain the exact nature of the ferment, if it is a ferment that causes fat necrosis.

THE NATURE OF THE SUBSTANCE IN PANCREATIC EXTRACTS THAT CAUSES FAT NECROSIS.—It remained to ascertain if the destruction point of the active substance in the pancreas corresponds to that of trypsin or of lipase as they exist in the preparations used. According to certain investigations, pure trypsin is destroyed at a temperature of 45° C. for five minutes in neutral solutions, but the presence of albumoses, proteids, and salts protects it to a considerable degree. The thermal destruction point of lipase seems not to have been extensively studied, but Duclaux says that its maximum action is produced at 60° C., the action at 70° C. being equal to that at 50° C. The thermal destruction point of the trypsin in the preparation chiefly used in this work was ascertained as follows: One gram of dry fibrin was placed in fifty cubic centimeters of 0.25 per cent Na_2CO_3, with five cubic centimeters of toluol, and to this was added one-half gram of Armour's pancreatin in ten cubic centimeters of water after the ferment solution had been kept for five minutes at the indicated temperature. The preparation was then kept in the incubator for several days at 35° C., the results being as follows:

		BIURET REACTION.		
		18 hrs.	*40 hrs.*	*72 hrs.*
1.........	Heated at 55°–60° for 5 min.,	+	++	++
2.........	" " 60°–65° " " "	faint	+	++
3.........	" " 65°–67° " " "	faint	+	++
4.........	" " 67°–70° " " "	trace	trace	+
5.........	" " 70°–72° " " "	—	—	—

The destruction point of trypsin in this particular specimen is, therefore, between 70° and 72° C. Specimens heated to 70° C. retain their tryptic action, but it is impaired. Here, therefore, is a close agreement between the destruction point of trypsin and that of the agent causing fat necrosis; but since, as the following experiments go to show, there is no pronounced difference between the destruction point of trypsin and lipase, this factor does not serve to settle the question.

Lipase[1] was found to be quite active in all forms of commercial pancreatins studied, and its degree of activity and thermal destruction point were studied by means of its splitting action on ethyl butyrate. A solution of Armour's pancreatin, containing one-tenth gram in ten cubic centimeters of water, was kept at various temperatures for five minutes. It was then cooled in cold water to room temperature, and to it were added one-fourth cubic centimeter of ethyl butyrate and one cubic centimeter of toluol. The mixture was then placed in the incubator in stoppered flasks. For controls, identical mixtures were used in which the pancreatin had been heated to boiling, since the best ethyl butyrate obtainable, as well as the pancreatin, possessed some acidity. The amount of splitting was determined by ascertaining the amount of acidity developed, by titrating against $\frac{m}{20}$ NaOH, with phenol-phthalein as an indicator.

		CC. $\frac{m}{20}$ NaOH.	
		75 min.	*20 hrs.*
1 . . .	43°–46°	16	
2 . . .	49°–50°	14	
3 . . .	54°–55°	14	
4 . . .	58°–60°	10	38
5 . . .	63°–65°	5	24.5
6 . . .	68°–70°	3	7
7 . . .	73°–75°	0.5	1.5
8 . . .	78°–80°	0.0	0.0

There is a steady decrease in lipolysis from about 55° C., with a marked falling off above 60° C. and practical cessation at temperatures over 70° C., which does not at all agree with the figures of Duclaux. The thermal destruction point, therefore, does not offer any aid in determining which, if either, is the active enzyme. Since there is a very close correspondence between the death points ascertained for both enzymes and for the causative agent of fat necrosis, the conception that one or both of these enzymes is concerned receives support.

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[1] A power to split ethyl butyrate was considered as indicating the presence of lipase. Certain French observers claim that this is not a proper indication, but they are contradicted by Hanriot.

The most natural method of learning which enzyme is responsible is, of course, the use of each ferment pure, but the difficulties attendant upon the preparation of pure enzymes make this impossible. As yet no method has been devised for isolating lipase from pancreatic juice or pancreatic extract. Trypsin, however, can be obtained more nearly pure since it acts upon the other two enzymes while suffering but little itself. A number of experiments were tried by this method. First (at the suggestion of Prof. L. B. Mendel) a preparation of Kuhne's pancreas powder was permitted to digest itself until lipolytic action was lost, and then injected. The two following protocols will show the method and results:

Protocol 122. *Cat injected with a solution of Kuhne's pancreas powder that was both lipolytic and tryptic. Death in twelve hours. Result positive.*

X. — 20 — '02, 4.30 P.M. Large yellow cat received intraperitoneally 10 cc. of a solution made as follows: 20 grams of Kuhne's pancreas powder were placed in 200 cc. of 0.2 per cent salicylic acid and left in the incubator 16 hours. The fluid was removed by squeezing in cheese-cloth and rendered neutral to phenol-phthalein with Na_2CO_3. This fluid, together with a small quantity of sediment that it contained, was used for the injection. It was very actively tryptic and fairly lipolytic, 2 cc. producing about the same effect on ethyl butyrate as 0.1 gram of Armour's pancreatin.

X. — 21 — '02, 8 A.M. Found dead and cold; could not have survived the injection more than 12 hours, and probably much less. Autopsy showed abundant but very minute typical foci of fat necrosis, not only in the abdominal but also in the subcutaneous fat. There were many ecchymoses in the mesentery and about the bladder, but little fluid and no other signs of acute inflammation.

Protocol 124. *Dog injected with a solution of Kuhne's pancreas powder that had lost all lipolytic power, but still proteolytic. Results negative.*

X. — 27 — '02, 3 P.M Injected into the peritoneal cavity of a small dog 20 cc. of a solution prepared as follows: The remainder of the extract used in experiment 122 was made alkaline with Na_2CO_3 to a strength of 0.25 per cent, thymol was added, and it was placed in the incubator for one week. The fluid was then found to be fairly active towards fibrin, although somewhat less so than when first used, but no action whatever was produced upon ethyl butyrate.

X. — 29 — '02, 3 P.M. Killed with chloroform, having been well ever since the injection. Absolutely no trace of fat necrosis found, nor any other evidence of the injection.

These experiments indicate clearly that tryptic digestion of pancreatic extract destroys its power of causing fat necrosis, and at the same time destroys its lipolytic properties. That the decreased action of the trypsin is the cause of the failure of the production of fat necrosis is improbable, since in the second experiment the increased amount of fluid injected more than made up for loss of trypsin; other experiments also exclude this factor. Similar results were obtained by permitting faintly alkaline solutions of Armour's pancreatin to digest themselves with thymol. For the first few days, while the lipolytic action was fairly strong, fat necrosis was obtained, but later, while the solutions were still actively tryptic, but had lost their lipolytic power, they did not cause lesions.

A preparation labelled "trypsin" was also tried. This substance was found to have a slight lipolytic action, but not more than a trace when compared with other "pancreatins." Its tryptic activity was about the same as the more active pancreatins; hence it differed from them chiefly in lipolytic strength. Injections were made into three animals, in doses of one-half gram, in the same manner as with pancreatin, but in none was fat necrosis produced.

As examples of trypsin-like enzymes that are not accompanied by lipase, the vegetable proteolytic enzyme of Carica Papaya was used. While Mendel[1] has showed that the end products of this enzyme are somewhat different from those of pancreatic trypsin, still the actions are very closely related. A preparation known under the trade name of "Caroid" and Merck's "Papain" were used, and found apparently identical in effect. They produced a most violent inflammation when injected into the peritoneal cavity, far beyond anything seen with trypsin, often causing death in a few hours unless very small doses were used. The peritonitis was characterized by much congestion and hemorrhage. With neither large or small doses could fat necrosis ever be produced. Not infrequently portions of hemorrhagic tissue were found necrotic, but the condition in no way resembled fat necrosis.

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[1] Amer. Jour. of Med. Sciences, 1902, cxxiv, 310.

Ingluvin was tried in one experiment, but it caused no changes. Investigation showed that it possessed neither lipolytic nor proteolytic powers.

Evidently trypsin and allied enzymes in various sorts of solutions cannot cause fat necrosis, even when there is also a slight lipolytic action. When the solutions are lipolytic enough, however, fat necrosis is produced. Is the lipase alone responsible, or is it a joint action? As before remarked, lipase cannot be isolated from the pancreatic juice. As a possible source of lipase, free from any considerable amounts of trypsin, Mr. A. S. Loevenhart kindly suggested the use of extracts of fresh hog liver and fresh cat serum. As before mentioned, the extract from the livers of hogs has been found by Kastle and Loevenhart to be at least twice as actively lipolytic as the extracts from the pancreas of the same animal. Hog's liver does contain an enzyme resembling trypsin, as shown by Salkowski, Jacoby, Croftan, and others, but it is in relatively minute amounts. Preparations were made by a method devised by Kastle and Johnson, as yet unpublished, which was furnished to me by Mr. Loevenhart; also simple extracts were made by grinding the liver with sand, and extracting with water one cubic centimeter for every gram of liver. The extracts in both cases were very actively lipolytic. Simple aqueous extracts were found to show as much lipolytic action per gram of liver used as one gram of Armour's pancreatin. These extracts were injected into the abdominal cavities and subcutaneous tissue of seven dogs and cats, often in large quantities, but no fat necrosis was ever obtained. The lipolytic strength injected in some experiments was as much as eighty times as great as in the doses of pancreatin that were certain to cause fat necrosis. In one experiment the liver extract was made up to one per cent Na_2CO_3, in another two per cent, but nothing except acute inflammation resulted.

The serum of cats is also strongly lipolytic, the action of one cubic centimeter being about the same as that of one-tenth gram of Armour's pancreatin. Twenty cubic centimeters of cat's serum was injected intraperitoneally into one dog, and ten cubic centimeters into another, and a little was injected subcutaneously into each; no fat necrosis resulted.

In all of these lipase experiments many times as much lipase was injected as in the experiments in which pancreatin was used, but without effect. Of course the great objection to these results is that there may be a difference between pancreatic lipase and the lipase of liver and serum. In fact, the studies of Kastle and Loevenhart render this more than probable. Whether this difference is of a nature to change its action in fat necrosis is, of course, problematical. The desirability of obtaining a pancreatic lipase without trypsin led to utilization of the results of Pawlow and his students. It has long been known that fresh pancreatic tissue, particularly from fasting animals, yields an extract that is almost entirely devoid of tryptic activity; it contains trypsinogen rather than trypsin. If the gland is kept several hours before extraction, or if an acid solvent is used, an active trypsin is obtained; that is, the trypsinogen is converted into trypsin. The Russian investigators have shown that a similar activation may be accomplished more effectively by adding to fresh pancreatic extract an extract of the intestinal mucosa; this action is accomplished by a ferment-like body that they call "enterokinase." As the lipolytic enzyme is present in fresh pancreas, and in full activity, it seemed worth while to see if the presence of enterokinase had any influence upon fat necrosis. It is to be noted that pancreatic extract may be actively tryptic when fresh, and the same applies to pancreatic juice, especially when it is obtained during digestion. This seems to be due to some substance, probably produced in the spleen, that energizes the trypsinogen before it is secreted, much as the enterokinase does after it has reached the intestine.[1] It is apparent that enterokinase does not play a part in producing ordinary fat necrosis, the pancreatic fluids reaching the fat tissue without coming in contact with intestinal juices. If the trypsin is at all concerned it owes its activity to the spleen, or the trypsinogen may possibly be converted after it has escaped into the tissues or peritoneal cavity, much as it does in glands that have been left for some time. The leucocytes may play some such activating rôle, for it has been found that extracts of the mesenteric glands produce much the same effect as the enterokinase of intestinal mucosa, and it has been thought by some that even the extracts of mucosa owe their activity to the contained leucocytes. If there is not some such change in the pancreatic juice, fat necrosis is ordinarily produced by pancreatic juice that is lipolytic and amylolytic, but not tryptic.

The experiments with fresh enterokinase and fresh pancreatic extracts gave interesting results. Extracts were made from the pancreas of dogs while the organ was still warm, by grinding the organ, then rubbing it up with sterile sand, extracting with two-tenths per cent Na_2CO_3, and straining through cheese-cloth. Such extracts were found to have a moderate lipolytic activity, 1 cc. producing about the same effect as one-tenth gram of pancreatin. If from feeding dogs they caused a very slight tryptic digestion, in no way equivalent to the pancreatins, but if from fasting dogs one cubic centimeter of the extract in ten centimeters of one-fourth per cent Na_2CO_3, with fibrin and toluol, would not cause the biuret reaction to be produced in the filtrate obtained after boiling, in twenty-four hours. Yet in each of two cats into which such preparations were injected a marked fat necrosis appeared.

Enterokinase solutions were made by scraping the mucous membrane from eight inches of the duodenum of a dog that had just been killed, grinding the pulp with sand, and extracting with water or two-tenths per cent Na_2CO_3. Such extracts showed no demonstrable tryptic reaction, although very slightly lipolytic; but when added to a perfectly inactive (tryptically) extract of dog's pancreas, they render it most remarkably proteolytic, digesting fibrin more rapidly than any of the commercial pancreatins. It is safe to assume, therefore, that when we mix intestinal and pancreatic extracts before injecting them into an animal we increase greatly the tryptic activity of the pancreatic extract, and hence any effect due to trypsin should be increased. Such mixtures were injected into four cats, in conjunction with the experiments cited just previously in which the pancreatic extract had not been activated, so that the experiments controlled each other. In not one of the four cats was a trace of fat necrosis found. Therefore we must conclude that enterokinase, or some other constituent of the dog's intestinal mucosa, destroys the power of extracts of fresh dog pancreas to cause fat necrosis in cats, while at the same time it increases the tryptic power of the extract. It is known that active trypsin quickly destroys the action of lipase, and it is most natural to assume that it is through this destruction of lipase that fat necrosis is prevented by intestinal extracts.

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[1] Mendel and Rettger, Amer. Jour. of Physiology, 1902, viii, 387.

An attempt to artificially combine the effects of lipase and trypsin was made by mixing actively lipolytic extracts of fresh hog's liver with the "trypsin" preparation before described, the latter being actively tryptic although very feebly lipolytic. Six experiments were performed on cats, using ten cubic centimeters of the liver extract, to which was added just before injection one-fourth gram of trypsin in three experiments, and one-half gram in the other three. In none was the slightest trace of fat necrosis produced.

From this series of experiments we are forced to conclude that trypsin alone cannot produce fat necrosis; and it has been equally impossible to produce fat necrosis with serum or liver extracts that were strongly lipolytic. When a pancreatic extract loses its lipolytic activity it loses *pari passu* its power of causing fat necrosis; likewise when its tryptic power is increased by enterokinase. These results have brought the experiments to an unsatisfactory conclusion, leaving the specific agent undetermined. It seems to me that one of the following explanations must hold:

1. There may be a third body, different from lipase and trypsin, destroyed by about the same degree of heat. This hypothetical body may either injure the fat tissue so that lipase can split the fat, or it may accomplish both effects alone.

2. Lipase alone may be the agent, the negative results of my experiments being due to a difference between pancreatic and liver and serum lipase.

3. Fat necrosis may be due to a double action, trypsin injuring the cells and lipase splitting the fat. The failure of the series of experiments directed to this point may be a mere mischance, or because the conditions produced in the experiments were not the same as those existing in pancreatic extracts. One such difference that is evident is the use of liver and serum lipase, rather than pancreas lipase.

CAN SUBSTANCES OTHER THAN FERMENTS CAUSE TRUE FAT NECROSIS?—Since the idea of enzyme reversibility did not agree with the facts known concerning fat necrosis, more particularly the presence of large amounts of fatty acids in the cells where normally fat exists, it seemed possible that some substance that interfered with equilibrium might be present, accounting for the continued splitting without synthesis. The pancreatic juice, in addition to the ferments, contains a considerable amount of alkali carbonate. Loevenhart has found that lipase cannot synthesize the ester from sodium butyrate and ethyl alcohol, and on investigation of the literature he found much evidence that in normal metabolism the fatty acids do not form soaps. Munck has showed that soaps are highly toxic. Sodium oleate, although less toxic than the palmitates and stearates, caused death of rabbits in doses of thirteen one-hundredths of a milligram per kilo when injected intravenously. On the other hand, free oleic acid is not toxic. Pflüger has shown that one per cent Na_2CO_3 saponifies the higher fatty acids very slowly and incompletely at 37° C., so that presumably there is but little chance of such a combination occurring in the body in a fluid of the alkalinity of the blood. However, it was quite natural to suspect that a solution as strongly alkaline as the pancreatic fluid might cause a union of alkali with fatty acid when freshly split, as in living fat. If this occurred, since the soap cannot be synthesized into fat by lipase, it would be impossible for equilibrium to be attained, and splitting would go on until no fat remained. This would at least account for the removal of the fat from the necrotic area, although it would not explain the presence of crystals of free fatty acids observed in the necrotic cells. Accordingly the effect of alkaline solutions upon the fat tissues was tried. One and two per cent Na_2CO_3 solution, sterilized in the autoclave, when injected intraperitoneally caused nothing at all comparable to fat necrosis, although it did produce a considerable degree of acute inflammation without bacterial aid. Pancreatic extract and pancreatin did not cause fat necrosis when boiled in a solution of one per cent Na_2CO_3 and injected with it, as mentioned previously, and weak acetic acid solutions were equally inert. Injection of two per cent Na_2CO_3 directly into the subcutaneous fat of dogs caused no necrosis, but a diffuse hemorrhagic extravasation with edema and leucocytic invasion. One per cent Na_2CO_3 containing either inactive pancreatin or " trypsin," injected into subcutaneous fat, caused an extensive inflammation with great leucocytic infiltration, but without any necrosis of tissue and nothing at all resembling fat necrosis. Strong solutions of NaOH naturally caused much greater changes. Injection of one cubic centimeter of a ten per cent solution subcutaneously in the tissues of a dog produced a large area of gangrene very quickly; in one hour the area was well defined, of a dark red color, with sharp borders, and on section the subcutaneous tissue was hemorrhagic and softened. Microscopically stained specimens showed that there is no sharp line of demarcation between the dead and living tissue. The fat suffers just the same as the tissue in which it lies. After twenty-four hours the skin is black and soft, but still intact; beneath it is undermined by liquefaction of the subcutaneous tissue, replacing which is an oily, purulent-appearing fluid. This fluid contains so many red cells after cutting into it that it cannot be well examined until cleared up with weak acetic acid. Then it is seen to consist largely of fat droplets of varying size, in and about which are many crystals of radiating clusters of needles, each individual crystal being shaped like an icicle. The crystals are found only where the red cells are being dissolved by acetic acid, and perhaps are the result of the action of acid on soaps. Sections taken from the edge of the zone of necrosis after seventy-two hours show in hardened and stained preparations irregular areas consisting of a group of fat cells full of a solid, eosin-staining substance. In every respect these areas resemble fat necrosis, except that they are not in as regular groups and are not so well demarcated. Leucocytes that appear normal are seen within the necrotic areas, and occasionally a line of leucocytes resembling the line demarcating areas of true fat necrosis can be found. Such pictures, which are infrequent, offer no distinct differences from the early stages of fat necrosis.

The possibility that the formation of calcium soaps played an important part by precipitating out fatty acids and destroying the equilibrium suggested that perhaps an alkaline calcium solution might cause conditions resembling fat necrosis. Subcutaneous injections of saturated solution of $Ca(OH)_2$ caused merely an acute inflammatory reaction (the solution was not sterilized) without alterations in the fat tissue, and without formation of any discernible accumulation of insoluble calcium salts after twenty-four hours.

As fat tissue contains lipase, the question arose as to the possibility of it by itself, causing the changes in the fat. To study this, portions of the omentum of dogs were shut completely out of all relation to the surrounding fluids and tissues by inserting a part of the omentum in a sterilized, thin rubber finger cot, and then deprived of nutrition by a ligature tied tightly about the base of the cot. In this way necrosis was produced in the fat, presumably without injury to any enzyme it might contain, without disturbing its chemical equilibrium, and without the influence of outside factors. Within three days the pieces sloughed off at the point of ligation and were found loose in the abdominal cavity, containing a soft and brownish fat. Sections of this fat showed it to be totally necrotic, with opaque, thick fibrils of connective tissue, and with but a few, abnormal nuclei surviving. The fat cells were completely empty in hardened and stained sections, and there was no resemblance whatever to fat necrosis. Frozen sections stained by sudan III. showed that the fat was still present in the fat cells, staining well, apparently in a perfectly unchanged condition

The part played by fatty acids in the necrosis was studied by placing lumps of sterilized palmitic acid and stearic acid in the subcutaneous fat tissue, and in a fold of the omentum in contact with fat tissue, where it was retained by a suture that formed a pocket. After two and four days the pieces with the surrounding tissue were taken out and hardened in formalin. Microscopic examination showed that the lumps of fatty acids had acted like perfectly inert foreign bodies, causing no changes but proliferation, and a slight leucocytic infiltration of the tissues immediately adjacent.

ACTION OF PANCREATIN POST MORTEM AND IN VITRO. — Not infrequently at autopsy necrosis of the fat immediately about the pancreas, and more particularly the fat lying between the lobules of the gland, has been observed.[1] At times cellular reaction has established the vital nature of the fat necrosis, but in other cases the necrosis is more diffuse and without cellular reaction, suggesting a change in the fat of post mortem origin quite analogous to the common self-digestion of the gland. After death the digestion of the gland destroys its consistence to such an extent as to readily permit of the escape of its fluids beyond its confines, and the question of the possibility of this pancreatic fluid producing fat necrosis after death becomes of interest. If fat necrosis is simply the reaction of fat tissue to certain enzymes it is reasonable to expect pancreatic extracts to cause the process after death, or even in vitro, if the temperature is kept favorable for enzyme action. The following experiments were performed:

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[1] Chiari, Zeitschrift für Heilkunde, 1896, xviii; also more recently Pforringer, Virch. Arch., 1899, clviii, 126; and Liepman, ibid, 1902, clxix, 532.

Experiments *in vitro:* Pieces of fat omentum were placed in a one per cent Na_2CO_3 solution containing pancreatin, and kept at body temperature in the incubator. At intervals portions were placed in formalin and examined histologically, both in frozen and imbedded sections. It was found that within half an hour the cells on the surface had lost their staining entirely, and the membrane surrounding the fat cells had assumed an increased affinity for eosin, and had become thickened. The appearance was exactly similar to the change seen in fat tissue in the earliest stages of fat necrosis (see subsequent description) before the necrosed fat cells became filled with the characteristic opaque material. The changes that follow from longer exposure simply consist of a loss of nuclear stain throughout the fat tissue, and later an entire loss of even the connective tissue structure.

POST MORTEM EXPERIMENTS.

Protocol 75. Injected 20 cc. of 1 per cent Na_2CO_3 solution containing 1 gm. of pancreatin intraperitoneally in a dog, immediately after it had been chloroformed to death. The body was left at room temperature over night. At autopsy there were found throughout the peritoneal fat many groups of small ecchymotic spots, about some of which were minute pale areas, barely visible, that suggested fat necrosis, but they were too small and indistinct to be positively identified as such. Sections of the omentum (in all cases in this work the tissues were hardened in formalin and imbedded in celloidin or frozen) show areas in which the fat tissue has lost its staining properties. Some of the fat cells in these areas contain finely granular material, more are empty, never is the amount comparable to that seen in the fat cells of fat necrosis. Occasionally the contents take the eosin stain, but never the hematoxylin. The areas are at the margins of the fat and in size and outline are much like foci of fat necrosis. In fact, the chief difference observable is in the small amount of granular material in the fat cells, absence of any blue stain, and absence of leucocytes.

Protocol 85. Dry pancreatin was sprinkled upon the anterior fold of fat tissue in the abdomen of a dog that had just been killed, and the fat tissue was folded over it. The body was sewed up and kept at room temperature for seventeen hours. Autopsy: At the place where the pancreatin was placed there was a soft cheesy mass of fat towards the surface; deeper in there were minute, barely visible opacities in the fat, too small to be identified. Sections showed that much of the fat, particularly on the surface, had lost its nuclear staining, and the fat cells contained a granular material which, in some instances, stained bluish, in others with eosin. In places this change is seen extending in a short distance from the surface, resembling fat necrosis strikingly. As a matter of fact there is no essential difference between these changes and true fat necrosis. The differences are of degree rather than of kind.

Protocol 117. Wads of cotton saturated with an emulsion of pancreatin were placed in the omentum, and held in place by sutures of silk, tied loosely to prevent interference with circulation. The dog was kept alive under anesthesia for one hour, and pieces were cut out at the end of one-half hour and one hour. The dog then died and the body was left at room temperature for eighteen hours. Sections of the tissue at one-half hour showed merely death of the peritoneum and some of the superficial fat cells, none of which contained any solid intracellular material. At one hour the necrosis had extended deeper into the fat tissue, without solidification of the contents of the fat cells. The sections of tissue removed after the body had been dead for eighteen hours showed that the outer layers of fat cells were necrotic, as in the other specimens; all of these and many deeper ones that were not necrotic were full of a solid exudate. It seemed no different from the intra-vitam reaction except that the process seemed to have extended somewhat since death with the development of the characteristic solid cell contents.

Protocol 78. Dry pancreatin was dusted over the omentum of a cat that had just been laparotomized for another purpose. Five hours later it was found that the abdominal wound, which had been sutured, had been ruptured and the omentum was strangulated through the opening apparently for some time, as it looked gangrenous. In some places in the omentum where it was not gangrenous were what appeared to be foci of necrosis, still very small and indistinct. Sections through the gangrenous part showed the entire surface to be necrotic, while the deeper cells appeared normal. It resembled ordinary necrosis of fat except that the surface cells were full of granular material resembling exactly the solid cell contents of fat necrosis. In other words, this tissue shows a mixture of simple necrosis of fat, and true "fat necrosis," which might be expected because of the conditions present, namely, gangrene and pancreatin. The sections look exactly like fat necrosis except in the diffuseness of the process and the absence of limitation or inflammatory reaction; on the other hand it looks exactly like the sections of *in vitro* digestion, except in the solid material that fills some of the superficial cells. It illustrates well the relationship of these processes.

These results seem to indicate that each of the processes that occur within the fat cells in fat necrosis may be produced experimentally outside the body, or within the body after death, with pancreatin. The typical anatomical picture is not reproduced, however, either macroscopically or minutely. The difference is always essentially one of degree rather than kind, suggesting that the characteristic grouping depends upon certain factors of circulation, or other processes inseparable from life.

STUDY OF THE SUCCESSION OF CHANGES DURING THE DEVELOPMENT OF FAT NECROSIS. — Previous experimental work, depending generally upon the extravasation of the secretion of the pancreas, has given little opportunity for study of the order of events that take place in the production of the typical foci of necrosis. The method of using pancreatin solution makes this very simple, and such study is of great interest. A number of experiments were performed as follows: A dog was anesthetized and the abdominal cavity opened aseptically; the omentum was drawn out, and at points separated as widely as possible small wads of cotton saturated with a five to ten per cent suspension of pancreatin in distilled water were placed within a fold of the omentum, and retained there by a suture, tied loosely to prevent disturbances of circulation. The omentum was then returned to the abdominal cavity and the abdominal walls were united by heavy through-and-through sutures, held together with artery forceps instead of by tying. The animal was kept anesthetized for the duration of the experiment, and at stated intervals the sutures were loosened, the omentum brought out, and a piece containing one of the cotton wads ligated off and removed, after which the omentum was returned to the abdominal cavity. The portions of fat in the removed piece of omentum that had been in contact with the cotton were at once placed in formalin for study. Sometimes the animal was permitted to come out of the anesthetic after a few hours, and killed twenty-four hours later to study the later changes, but generally it was chloroformed to death after five or six hours. Several such experiments were performed, and the results were practically the same in all, the differences being but trivial and chiefly quantitative. From them the manner of development of the typical focus of fat necrosis was found to be about as follows:

Fifteen minutes. — The changes at this time consist of a general engorgement of the capillaries between the fat cells, with at places hemorrhages. (The amount of hemorrhage varies greatly in different cases, and as it is much more abundant in these experiments than in simple injection experiments, probably much of it is due to the unavoidable manipulation of the omentum.) The peritoneal endothelium is swollen, and in many places necrotic, as shown by a loss of visible nuclei, while the cytoplasm becomes granular and often distintegrated. Where the endothelium is destroyed the underlying connective tissue is frequently swollen, the fibrillar outlines obscured, and the eosin staining increased in intensity.

Thirty minutes. — The changes noted at fifteen minutes have become more extensive, and in a few places the fat cells nearest the affected surface have lost their nuclei, and have undergone changes in their walls indicating necrosis. There is a slight leucocytic infiltration beginning throughout the fat tissue. The necrotic fat cells are entirely empty in hardened sections. In frozen sections they contain what appears to be normal fat, staining by sudan III., and without crystals.

One hour. — Many of the small vessels contain hyalin thrombi, and the infiltration of leucocytes has become more extensive. There are more necrotic cells than at thirty minutes, but they still contain normal-appearing fat, without crystals. Where the areas of hemorrhages are near the surface, when the surface is necrotic, the red cells have lost their outline and appear as a homogeneous yellow mass.

Two hours. — The changes have increased only in degree, with the exceptions that the leucocytes have to a considerable extent migrated to the margins of the areas of necrosis, and in some places form a wall about part of the area.

Three hours. — By this time the accumulation of the leucocytes at the boundaries of the necrosed tissue have become more dense, and many of the areas are well walled off, so that the resemblance to typical fat necrosis is readily seen. About this time is first seen the presence of an eosin-staining material in a few of the most superficial fat cells, which has not been dissolved out by the alcohol and ether used in imbedding in celloidin. Some of the cells are completely filled with this substance, while others but partly so; no crystals can be found in this solid substance. In frozen sections the contents of these superficial cells are opaque, structureless, and no crystals are present; when stained by sudan III. this material stains a faint yellow, in some cells not at all, with a few interspersed fat granules that stain deeply. The cells farther from the surface contain fat that stains well. Areas of hemorrhage, which are marked in some instances and slight in others, show considerable disintegration of the erythrocytes with formation of pigment, especially when the tissue between the blood and the surface is necrotic.

Four hours. — There are no essential differences between the condition at this time and at three hours, except that because of more extensive solidification of the contents of the fat cells and more demarcation by leucocytes, the picture of true fat necrosis is often completely produced. I have never been able to find crystals as early as this. In some four hours specimens can be found in fat cells located at the margin of the encompassing wall of leucocytes in which solidification has taken place, where the solid material takes a blue stain with hematoxylin about the same in intensity as ordinary nuclear staining. The blue staining substance seems to form in fume-like projections from the wall of the fat cell, often enclosing clear spaces in the meshes it forms. This seems to mark the earliest stage in the formation of calcium salts, and from its location at the edge of the necroses it seems due to union with calcium salts diffusing in from the outside.

Five hours.— By this time the necrosis is extensive, and most of the fat cells that have been in immediate contact with the cotton are necrosed for a depth of from one to three rows, often deeper in places. The various stages described are merely the maximum advance for that length of time; the slighter degrees are also present in the same specimen at the same time. Occasionally single foci, consisting of but one or two fat cells that are necrotic and walled in by leucocytes, are seen deep in the tissue, and, as shown by serial section, in no way connected with the surface; sometimes these isolated areas have no evident relation to the vascular structures, but not infrequently they are on the margins of large lymph spaces, which are usually widely dilated during this inflammatory process. Rarely at this time are found fatty acid crystals in frozen sections, and still more rarely in celloidin preparations. After six hours they generally become quite abundant. When they are present in frozen sections they can usually be found in the celloidin preparations, but always in much smaller numbers.

From the sixth hour on the changes vary greatly in different cases, depending on factors to be discussed later, but by this time the production of true and typical fat necrosis has been accomplished. To summarize these studies the changes in the evolution of focal fat necrosis are as follows:

First the structures in immediate contact with the pancreatin suffer, and necrosis of the peritoneal endothelium and the underlying connective tissue may occur in a very few minutes. The process seems to extend by contiguity into the fat cells nearest, and they die as did the peritoneal connective tissue. At the same time the reaction of acute inflammation goes on, and leucocytes migrate from the vessels, both deep and superficial. They tend to accumulate about the areas of necrosis, and by the end of three hours there is usually a distinct walling off. About this time the contents of the fat cells that are nearest the surface from whence the process is extending undergo a change whereby their contents become solidified, the resulting substance being so little soluble in cold alcohol and ether that it passes through the ordinary process of celloidin imbedding; this substance does not stain by sudan III., and it seems to entirely replace the fat, so that when the process has filled the cell there can be found at the most but a few minute fat granules in the affected cell. Crystals that can be recognized as fatty acids have been found first about the fifth hour, but they usually are not abundant until a few hours later; they also escape solution in alcohol and ether, at least in part, and they seem to replace the solid, non-crystalline substance that first replaces the fat. After the fourth hour a basic staining substance begins to appear in some of the cells that are solidified and nearest the still intact portions of tissue.

The subsequent changes vary greatly. In ordinary cases the amount of necrosis does not increase much after about the eighth hour, and it may stop even earlier. What will follow after this will depend entirely upon the conditions that exist. If a large amount of very active pancreatin is brought into the peritoneal cavity the areas of necrosis will be much larger than when the amount is smaller, and the healing will take place with corresponding slowness. It is often possible to find healing as far advanced after forty-eight to seventy-two hours in some instances as it is after eight to ten days in others. The process seems to be as follows: After the area of necrosis has been fairly well walled off by leucocytes further extension does not occur, and the process from that time on is essentially a healing one. The leucocytes nearest the surface suffer much karyorhexis, and often large masses of nuclear detritus are formed within and between the fat cells. The solid material that fills the fat cells seems to be fatty acids that have not crystallized. This is based upon the fact that they do not stain with sudan III., but replace the fat without observable intermediary steps or infiltration from outside, and that later they seem to be directly replaced by the crystals whose structure shows them to be fatty acids. One observation that is difficult to explain, particularly on this basis, is that the solid fat cells are larger than those about them and appear to be swollen; this is possibly due to the looser formation of the fatty acids than the fats. Calcification, if the presence of the blue-staining material is to be so interpreted, proceeding as it does from the intact tissues, is more complete and earlier in small areas. In minute foci all the cells may be full of the blue-staining material within twenty-four hours, while with large areas this substance may be found only about the edges, and never extend into the center of the foci. The amount of leucocytic invasion varies — when the foci are small the infiltration is usually great and rapid, and anything that increases the irritation, as handling or infection, seems to increase their numbers. Within twenty-four hours the small foci may be so filled with leucocytes that almost nothing can be seen of the cell contents, which have been replaced by leucocytes and their debris; oftener it requires seventy-two to ninety-six hours to produce this condition. By the end of forty-eight hours there is a noticeable proliferation of new connective tissue cells at the margin of the foci, and this varies at first inversely with the amount of leucocytic invasion; with the large foci healing is chiefly by proliferation. These new cells are large, spindle or oval, and when they have formed a considerable band about the focus of necrosis the leucocytes are found chiefly within it, apparently accomplishing absorption of the dead fat cells and their contents. (It was this zone of new connective tissue that Balser thought represented new-formed fat cells, and to which he attributed the production of necrosis through their shutting off blood supply.) Giant cells were never seen. Occasionally leucocytes were seen partly surrounding the ends of crystals, resembling phagocytosis, and phagocytosis of nuclear fragments was frequent. Eventually the tissue is entirely replaced by the new connective tissue containing new vessels, and the peritoneum heals over with endothelium, rarely forming adhesions. Fibrinous exudate is not usually present on the surface of areas of necrosis unless there is infection.

The areas of hemorrhage bear no particular relation to fat necrosis; sometimes they become necrotic and sometimes not. When they do become necrotic the red blood corpuscles soon lose their form and a diffuse yellow mass results, with some pigment. Within three or four days the only trace left of blood is a great amount of yellow granules of pigment in areas which otherwise resemble typical fat necrosis.

The nuclear changes that occur in the cells that become necrosed are chiefly those of karyolysis, but it is quite different with the invading leucocytes, which become fragmented, often to a great extent. The small spherical granules that are left take a very intense stain, almost black, suggesting the possibility that the trypsin has split off the proteid portion of the nucleo-proteid, leaving the densely-staining nucleic acid by itself.

DURATION OF FAT NECROSIS.— The length of time that the foci exist visible to the naked eye within the abdominal fat will evidently vary greatly with the size of the lesions. When they are small they disappear very quickly, as is shown by the following experiment:

Protocol 80. VII. — 1 — '02. Injected into the abdominal cavity of a dog 10 cc. of water containing 0.5 gram of a preparation of pancreatin that was not very active. After the first day the dog seemed well.

VII. — 5 — '02. Opened the abdomen aseptically, under chloroform anæsthesia. In the fat of the fold of peritoneum that lies in the anterior median line of the dog's abdominal cavity were found perhaps a dozen typical foci of fat necrosis, none larger than half the size of a pin-head. A portion of the fat tissue was ligated off and removed for study. It showed typical foci, partly replaced by young connective tissue, and invaded throughout by leucocytes.

VII. — 12 — '02. Killed by chloroform. Absolutely no trace of fat necrosis could be found. A piece of tissue removed from the proximal side of the ligature, where there had been areas of necrosis seven days before, and examined histologically, showed a number of small foci of hyalin connective tissue, containing many small blood vessels and numerous large connective tissue cells.

In this case healing had been so complete in eleven days that nothing was visible to the naked eye, but the original foci had been minute. On the other hand, when large areas are produced the healing changes may not be very far advanced in eight or ten days, and it would probably be several weeks before they would be invisible, if ever. There are many cases in human beings where foci were still abundant at autopsy in which death has occurred at much greater intervals after an operation at which the fat necrosis was detected than in the experimental animals. However, in these cases the pancreatic juice was escaping more or less continuously, and the lesions found at autopsy may have been formed later than those seen at operation.

Fat necrosis, *per se*, seems to be of no great moment as regards the life of the subject. It is not the fat necrosis that causes the symptoms or the fatality, but the lesions of the pancreas that produced the fat necrosis, or the resulting shock, peritonitis or gangrene. Fat necrosis is merely one of the results of escape of pancreatic juice from the gland, and means nothing more to the surgeon than that such escape has occurred. As has been observed by surgeons, a patient may recover from extensive fat necrosis without anything having been done to the pancreas itself. An animal may have extensive fat necrosis and yet appear perfectly well.

TIME OF APPEARANCE OF FAT NECROSIS. — A few human cases have been observed in which it was possible to note that extensive fat necrosis could be produced within a comparatively few hours. H. Marx[1] reports a case in which laparotomy was performed upon a case of hemorrhagic pancreatitis, and no traces of fat necrosis found. Death occurred twenty-seven hours after the operation, and at autopsy, six hours after death, there was abundant fat necrosis, "areas the size of a lentil" being found. Marx also quotes a case reported by Simmons in which similar findings were made at operation and at autopsy, thirty-six hours apart. A few experiments were made upon animals to determine the minimum time. Since experiments showed that changes were visible microscopically as early as fifteen minutes after application of pancreatin, and distinctly recognizable fat necrosis was present at the end of three hours, it was merely a question of how early the changes are in sufficient amount to be visible to the naked eye as the typical sub-endothelial white spots. The experiments made with wads of cotton could not be used for this purpose because there was so much hemorrhage and hyperemia as to obscure the early changes, besides not being at all comparable to the ordinary conditions of fat necrosis. However, even here there could be seen at the end of four hours a number of minute white streaks that were highly suggestive of fat necrosis.

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[1] Virchow's Archive, 1901, clxv, 290.

One experiment consisted of placing a large lump of cotton saturated with five per cent emulsion of pancreatin upon the omentum, and examining the omentum under it at frequent intervals, the animal being anesthetized. In something less than three hours and twenty minutes a few very small foci could be seen. Of more value was the following experiment: Ten cubic centimeters of a one per cent Na_2CO_3 solution containing one-half gram of pancreatin was injected into a dog. After five and one-half hours it was killed with chloroform, and abundant small foci were present in the omentum opposite the point of injection, and a few small areas in the mesentery. Histologically there was found abundant typical fat necrosis.

In another dog that died about twelve hours after injection very extensive necrosis was present throughout all the abdominal fat, and even in the pericardium.

It would seem that the areas become visible to the naked eye when the solidification of the contents of the foci renders them opaque and white, which is generally about four hours after the pancreatin comes in contact with the tissues (in dogs and cats).

MANNER OF EXTENSION. — Fat necrosis is found not only upon the surface of fat tissue lying in contact with the peritoneum, but the areas may be found deep within the intra-abdominal fat tissue; furthermore they may be found outside the abdominal cavity, in the pericardial and mediastinal fat, and in the subcutaneous tissue. This has been observed in the human cases and also in the domestic animals. As it is difficult to understand the relation of the escape of pancreatic juice to such remote lesions, some have thought that this remote process may be of a different nature. E. L. Opie[1] found, however, that equally widespread fat necrosis could be produced experimentally in cats after the pancreatic ducts had been ligated for twenty days or more. As in human cases the distribution was chiefly in the pericardium and subcutaneous fat, but foci were also observed along the carotid and subclavian arteries. Animals that survived but a few days did not show fat necrosis outside of the abdomen, except one animal which received pilocarpin after the ducts had been ligated, in order to increase the pancreatic secretion, and which showed foci in the pericardial fat four days later. Opie hardly explains the way in which the agent causing fat necrosis reaches the pericardial fat, and the fat about the great vessels in the neck. He observed that the process occurred only in animals whose pancreatic duct had been ligated for long periods, and that the foci in the pericardium showed a much younger state than those in the omentum. Also the process was less extensive more remote from the pancreas. Hence he speaks of the distant parts as being perhaps "reached by gradual diffusion through continuous layers of connective tissue." With this supposition I can hardly agree. In the first place, the intra-thoracic and subcutaneous foci, however numerous, are separated by considerable amounts of fat tissue that is intact. Again, I have often observed small foci deep in the omental fat, entirely separated from the surface by fat microscopically normal. Neither is any great length of time required to produce extra-abdominal foci, as is shown by experiments, of the protocols of which the following are brief abstracts:

Protocol 50. Dog received 50 cc. of an emulsion of fresh hog's pancreas in 0.2 per cent acetic acid intraperitoneally. Death occurred about *twelve hours* later. Foci of fat necrosis abundant throughout the abdominal fat, and in the lower part of the pericardial fat were a few small areas.

Protocol 60, 56. A cat which received intraperitoneally 10 cc. of emulsion of fresh hog's pancreas in 1 per cent Na_2CO_3 died after about

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[1] Contributions to the Science of Medicine by pupils of Wm. H. Welch, 1900, 859; also Johns Hopkins Hosp. Reports, 1900, ix, 859.

eighteen hours. Moderate amount of fat necrosis in the abdominal fat, and in the lower part of the pericardium a few foci were found, but there were none above the heart.

Protocol 60. Received intraperitoneally 20 cc. of an emulsion of fresh hog's pancreas in 0.4 per cent acetic acid. Killed after *eighteen hours*. Fat necrosis was distributed throughout the abdominal fat in moderate amount, and a few foci were found in the lower part of the pericardium.

Protocol 101. A small cat received intraperitoneally 10 cc. of an emulsion containing 0.5 cc. of pancreatin (Truax, Greene & Co.) in water. Killed *twenty-four hours* later, and an enormous amount of fat necrosis was found in the abdomen, in addition to which abundant foci were found all over the pericardium and in the fat above it as high as the clavicles, along which were a few spots. There were no foci along the ribs, but there were a few in the posterior mediastinal fat.

Protocol 92. Injected into a dog 10 cc. of water containing 0.5 gm. pancreatin. Killed after *two days*. In addition to a very extensive fat necrosis throughout the abdominal cavity there were a few foci in the pericardium, but none elsewhere.

Protocol 77. A large cat received 5 cc. of water containing 0.25 gm. of pancreatin. Killed after *nine days*. Most extensive fat necrosis throughout the abdomen, many typical foci in the subcutaneous fat from the costal arch to the umbilicus, several large foci on the pericardium as high as the base of the heart, but none above that point. Extending along the ribs, beneath the pleura on each side, are rows of small foci, most abundant about the middle of each thoracic wall, although there are many as far back as the costo-vertebral junction.

If one considers the distribution of these lesions, especially as seen in 77 and 101, and in the human case described by Chiari, and in the experiments of Opie himself, the probability that the path of dissemination is the *lymphatics* is strong. In what other way could such a localization occur without involving all the intervening tissue? As will be noticed, all the foci are found along the course of the lymphatic channels, particularly the beadings between the ribs along the course of the intercostal vessels. In just such a way is miliary tuberculosis spread, with the bacilli localizing their action at various points along the lymph vessels, favored by local stagnation or obstruction. In favor of this view is the following observation: In the fat below the surface of the omentum in the experiments performed to show the sequence of changes, there were frequently observed minute foci of fat necrosis appearing about five hours after the process had been started, involving at first but one or two cells, but later spreading so that they sometimes formed foci of a size readily visible to the naked eye. While in their earliest stages it was possible to determine that the starting point was a cell in contact on one side with a lymph space, which was readily seen as all the lymphatic channels were greatly distended because of the inflammatory process. As there could be found in serial sections no other way for the necrotizing agent to pass from the surface to these areas, it seemed probable that the lymph had brought it from the eroded surface.

Vascular transportation is improbable because of the location of the secondary foci. One experiment only was made with intravascular injection, with entirely negative results. Five cubic centimeters of water containing one-fourth gram of pancreatin was injected into the femoral vein of a medium-sized dog. On the following day the dog appeared somewhat weak, but not in bad condition, and after twenty-four hours it was killed. Autopsy disclosed no lesions in the body except inflammation at the point of injection.

The peculiar character of the lesions themselves, their formation in minute foci like miliary tubercles, is another characteristic of the process. This seems to be due entirely to local conditions. The way the omentum and intestines lie in the abdominal cavity, it is evident that any injected or extravasated fluid must lie in small nooks and crannies, and thus come in contact only with small areas of fat, which alone suffer. In proof of this is the fact that when the amount of injected material is large and active, so that a large surface of omental fat comes in contact with it, the entire surface necroses and a band of friable white fat is seen that bears no resemblance to the more common focal lesions except in color and consistence. In fat necrosis produced by placing wads of cotton saturated with pancreatin in contact with fat tissue, the necrosis occurs chiefly in lines and masses on the surface in contact. When fat necrosis arises from pancreatic lesions the omentum and peripancreatic fat nearest the gland often form one necrotic mass, while farther away, where the fluid is present in smaller quantity, and thus not affecting large surfaces, the focal character is seen. In experiments upon dogs in which a feeble solution was used so that the resulting foci were few in number, they were usually almost entirely limited to the fold of fat that lies along the anterior surface of the peritoneum, where the injected fluid naturally gravitates in these quadrupeds.

The earlier writers found that in human fat necrosis the lesions tended to be limited by the normal septa of the fat, confining it to individual lobules, and they considered this lobular character of some importance. It was found in these experiments that when spreading fat necrosis reached connective tissue septa it stopped there, although the connective tissue was often somewhat involved in the necrosis. However, the necrosis bears no relation to the septa further than this, and the septa only form the boundary when the necrosis spreads sufficiently to reach them; small foci are bounded by intact fat cells or by leucocytes.

SUMMARY.[1]

1. Fat necrosis seems to be merely a special form of necrosis of fat tissue, differing from simple necrosis chiefly in the sharp limitation of the affected area, usually by a wall of leucocytes and later by connective tissue; and the filling of the necrosed cells by the products of fat splitting. Each of these features can be produced experimentally in various ways, but the complete picture has never yet been produced except by products of the pancreas.

2. Fat necrosis can be produced with constancy in cats and dogs, less successfully in rabbits, by intraperitoneal injections of extracts of fresh hog pancreas, and nearly as well with dog pancreas. The results are the same with solutions that are in weak alkalis (Na_2CO_3), weak acids (NH_3COOH), or in water. Fat necrosis produced in this way is the same in appearance, both macro- and microscopically, as human fat necrosis.

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[1] It is to be borne in mind that these conclusions are based on results obtained with experimental animals alone.

3. Equally constant results can be obtained with ordinary commercial "pancreatins."

4. Preparations of Carica Papaya, although highly irritating, do not produce fat necrosis.

5. This property of pancreatin to produce fat necrosis survives heating for five minutes at a temperature as high as some point between 65° and 71° C.; above this point the property is entirely lost. The amount of fat necrosis produced decreases steadily after exposure at 55° C. and upwards. These observations point to enzyme action as the source of the condition.

6. It has been impossible to ascertain which of the pancreatic enzymes causes fat necrosis. Trypsin, weak in or devoid of lipase, will not produce this effect. Lipolytic extracts of hog's liver or cat's serum are likewise inactive. Mixtures of lipolytic extracts of liver with pancreatic trypsin will not produce fat necrosis. Extracts of fresh dog pancreas that are, when injected, feeble in or devoid of tryptic power, but possess lipolytic power, cause fat necrosis. If to such extracts an emulsion of duodenal mucosa is added, the enterokinase greatly increasing the tryptic activity, no fat necrosis will be produced. As the lipase of pancreatic extract cannot be isolated, it is impossible to ascertain if it by itself is capable of causing fat necrosis; but it seems highly probable that it is essential. It may be that the lipase causes fat splitting after some other ingredient of the pancreatic juice has injured the cell.

7. When fat tissue within the body has been made necrotic and preserved from outside influences of absorption, etc., the lipase that it may contain does not produce the changes of fat necrosis.

8. Simple alkaline solutions of the strength of pancreatic juice or slightly stronger (NaOH, $Ca(OH)_2$, Na_2CO_3) do not produce fat necrosis.

9. Many of the features of fat necrosis may be produced after death in animals, and also *in vitro*, with pancreatin, but the resulting condition does not resemble fat necrosis at all closely.

10. Dissemination outside the abdominal cavity has been observed as early as twelve hours after intraperitoneal injection. The route by which the spreading is accomplished is probably the lymphatic system.

11. The forms of the foci produced seem to depend simply upon the area exposed to the action of the pancreatin.

12. The earliest change in fat necrosis is a simple necrosis of the surface tissue, which extends gradually into the deeper fat cells. Fat splitting is subsequent to the necrosis and not its cause. At first the products are non-crystalline, but become so later.

13. The process progresses for but a few hours at any one point, the extension seeming to be limited by surrounding leucocytes. Absorption of the area is accomplished by leucocytes, and healing is by proliferation of connective tissue from the margins. Adhesions are seldom formed.

14. The foci become visible to the naked eye in three to five hours. They may disappear within eleven days, or persist for a much longer time, depending chiefly upon their size.

15. Fat necrosis by itself is not dangerous to the affected animal, and may develop while the animal shows no observable symptoms.

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OBSERVATIONS ON THE MORPHOLOGY OF B. DIPHTHERIÆ, B. PSEUDO-DIPHTHERIÆ, AND B. XEROSIS.*

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INTRODUCTION.

In 1887 Hoffman-Wellenhof[1] called attention to a bacillus found in the throat which resembled the diphtheria bacillus, but was shorter, thicker, and without virulence. Independently in the same year Loeffler[2] described the same bacillus, which was considered by him also to be a different organism and called the "pseudo-diphtheria bacillus." In 1890 Roux and Yersin[3] asserted that the differences described by Hoffman and Loeffler were not sufficient to separate the two species, and that the pseudo-diphtheria bacillus merely represented a non-virulent diphtheria bacillus which might under certain conditions assume virulence. These two opposite views are held to this day.

In 1893 C. Fraenkel[4] called attention to the resemblance of B. xerosis to B. diphtheriæ, and suggested the possibility of their being identical. Bacillus xerosis had been described in 1883 by Neisser and Kuschbert[5] as the cause of the rare disease, "Xerosis conjunctivæ." It has since been found that B. xerosis is a common inhabitant of healthy conjunctivæ and has no connection with the disease. The identity of the B. xerosis and B. diphtheriæ has also been claimed by Schanz,[6] Peters,[7] and others.

Among the points of differentiation between these three species the following have been suggested:

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* Read at a meeting of the American Public Health Association (Laboratory section) Dec. 8, 1902. Received for publication Jan. 8, 1903.

Acid production in dextrose bouillon. — B. diphtheriæ is a marked acid producer. B. xerosis also produces acid, though less. B. pseudo-diphtheriæ produces no acid; on the contrary, the bouillon becomes more alkaline.

Appearances of growth in bouillon. — B. diphtheriæ grows at the surface, with a flocculent sediment, leaving the bouillon clear between. B. xerosis is similar, but the sediment has a characteristic granular appearance. B. pseudo-diphtheriæ causes clouding of the bouillon.

Neisser's method of staining, when carried out under the conditions laid down by the author,[8] shows typical granules in B. diphtheriæ, but not in B. pseudo-diphtheriæ or B. xerosis.

Animal inoculations controlled by antitoxin have been advocated by Spronck[9] who found that B. diphtheriæ and B. xerosis produced slight local lesions — induration, edema, etc. — at the point of inoculation similar to the lesions produced by diphtheria bacilli of slight virulence. If antitoxin was inoculated with the cultures, he found that the lesions produced by the slightly virulent diphtheria bacilli were prevented, while with B. pseudo-diphtheriæ and B. xerosis the lesions were not affected.

The agglutination test has been used by Lubowski[10] as a means of differentiation. The technique for the agglutination of the diphtheria bacillus is necessarily so complicated as to impair the value of this test.

Impression preparations of six-hour colonies have been found by Neisser[8] to give characteristic appearances.

These points of distinction have not been found sufficient by all observers. Moreover, methods of differentiation which require the isolation of the bacilli in pure cultures take too much time to be of practical value in diagnostic work. Diagnosis must be based on morphology, and it is therefore especially important to be able to distinguish these organisms morphologically. Considerable work has recently been done along these lines.

Kurth[11] has again emphasized the characteristics originally described by Hoffman and Loeffler, namely, the length oı the bacilli, as a means of separating B. diphtheriæ and B. pseudo-diphtheriæ.

Wesbrook[12] has devised a classification of the different morphological types of B. diphtheriæ which may be used as a basis for recording the results of examinations, and which can materially simplify the discussion of the subject. With the use of Wesbrook's charts and plates a committee of the Massachusetts Association of Boards of Health[13] has carried out extensive co-operative investigations in regard to the frequency of the different types of bacilli in the noses and throats of healthy persons.

Wesbrook and Gorham[14] have noted that during convalescence from diphtheria there is a tendency for the bacilli in the cultures "for release" to change from the granular to solid forms like B. pseudo-diphtheriæ. Gorham interprets this change to be the result of the action of the body fluids of the immune individual, under the influence of which the bacilli both lose their virulence and become altered in form.

Wesbrook, Gorham, Ohlmacher,[15] and a few others have found that the short, solid forms of the bacilli, morphologically identical with the forms described by Hoffman and others as B. pseudo-diphtheriæ, have virulence for guinea pigs. Westbrook has found these forms alone in cases of clinical diphtheria.

Ohlmacher has found that the short, solid, virulent forms may be transformed into granular forms, as, for instance, where solid forms have been inoculated in a guinea pig with fatal result, granular forms may be recovered in cultures from the site of the inoculation.

These observations would lead to the conclusion that the diphtheria bacillus is pleomorphic; that is, that the same bacillus, producing the same toxin, has different forms when growing under similar conditions.

The question of the occurrence of pleomorphism in bacteria is an important one. By pleomorphism is not meant the variations in form which bacteria show when growing on different media or under other varying conditions. Nor do such changes as take place in the different stages of development of bacteria constitute pleomorphism. According to Migula,[16] pleomorphism in this restricted sense does not occur in the lower bacteria.

Zopf,[17] who has many followers, claims that we must admit the occurrence of pleomorphism if we include the groups of the higher bacteria — leptothrix, cladothrix, and streptothrix. Migula, however, points out that most of the variations seen in these higher bacteria represent different stages in the development, or result from varying conditions of growth.

It has seemed to the writer that the diphtheria bacillus is pleomorphic, if at all, only in so far as it has a stage of development like that of the higher bacteria, and that most of the changes in form represent merely different stages in its development, or result from varying conditions of growth. The work here reported was undertaken to determine what are the changes in morphology which take place in the diphtheria bacillus and allied forms during their growth on blood serum, and also what are the effects on their morphology of certain variations in the conditions of growth, such as temperature, reaction of media, and symbiosis.

The development of the diphtheria bacillus is so rapid that within the first fifteen hours the bacilli may undergo marked changes in their morphology. These changes can only be appreciated by the study of preparations made at intervals from the same culture, beginning after a few hours of growth.

The descriptions of the morphology of bacteria usually given in the text-books are based on the appearance of twenty-four-hour cultures incubated at 36° C., with a mention of the appearances in older cultures. The descriptions of the diphtheria bacillus are of this sort, and give the student no idea of the different forms which may be met in the course of their development. One object of this work has been to put on record, chiefly by photo-micrographs, a description of the development of the diphtheria bacillus in serum cultures.

The results which are here reported are based on observations of mixed and pure cultures made in the course of routine work at the Brookline Board of Health Laboratory during the past three years, together with the results of the systematic study of about fifteen hundred special preparations which have been made during the past six months in the manner to be described.

BACILLUS DIPHTHERIÆ. — Ten pure cultures of B. diphtheriæ from clinical cases, and of proved virulence to guinea pigs, were studied. Emulsions of a small amount of growth of twenty-four-hour serum cultures were made in distilled water. Serum tubes were inoculated by rubbing a loopful of this emulsion thoroughly over the surface. The culture was incubated at 36° C., and preparations were made from each at the end of four, eight, twelve, fifteen, twenty-four, thirty-six, and forty-eight hours. Different parts of the surface of the serum were used for each preparation, and care was taken not to use parts close to the water of condensation or the dried area at the top. The morphology of the bacilli in each preparation was carefully noted.

The accompanying plates will serve to illustrate most of the points mentioned in the following descriptions of the bacilli at different ages and under varying conditions.

Four-hour cultures. — There is no visible growth on the serum at this age, but many bacilli are found in the preparations. The bacilli are solid staining forms, frequently appearing to be paired (C^2 and D^2).* There is considerable variation in size and shape, some rods being decidedly clubbed. The irregularity at this age is probably to be explained by the fact that these bacilli are daughter cells of the club-shaped bacilli in the twenty-four-hour culture from which the tube was inoculated. This irregularity is even more marked in two and three hour cultures, and becomes gradually less with continued cell division.

Eight-hour cultures. — The growth is usually just visible. The bacilli here also are solid staining, more commonly arranged in pairs parallel to each other and of more uniform size than in the four-hour cultures. Occasionally the ends of the bacilli stain faintly and small granules may appear, but the granules are not common in pure cultures at this age except where a very vigorous growth has become apparent on the surface.

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* These letters, here and in other parts of the paper, refer to Wesbrook's types.

Twelve-hour cultures. — The visible growth at this age is usually marked. Microscopically the bacilli are longer. Many of them stain faintly toward their ends, and often at the tip of the lightly stained portion a small deep granule is seen. In other cultures the granules appear in short, even-staining forms with rounded ends. As a rule, however, the typical granular forms (C and D) are not common in pure cultures of this age.

Fifteen-hour cultures. — The changes noted above have become more marked. The bacilli are still longer and occasionally larger at one end (clubbed). The granules are larger and more abundant. The solid staining forms are relatively fewer.

Twenty-four-hour cultures. — At this age the solid staining forms are very few in number or wholly absent. The bacilli are longer. The paired arrangement may still sometimes be seen, but the individuals of the pair are both much longer and swollen at their distal ends (clubbed). Most forms show granules; the long clubbed forms present small granules, while the short bacilli of even contour have larger ones. Instead of granules, some of the bacilli may show protoplasm broken up into segments, giving a barred appearance, although seldom so regular as the barred forms of B. xerosis.

Thirty-six-hour cultures. — The bacilli are longer, and the appearances noted in the twenty-four-hour cultures have become more marked. Many of the bacilli may begin to take the stain poorly.

Forty-eight-hour cultures. — Many of the bacilli stain poorly. Occasionally, where the colonies are not confluent, the bacilli may grow out into even longer forms (filaments).

The age of the cultures at which these different changes take place varies considerably. Thus at times granular forms may be present in eight-hour pure cultures, while again their appearance may be delayed till fifteen hours. The nearness of the colonies has a marked influence. When the colonies are confluent the granular forms appear earlier than when the colonies are discrete. Different strains of B. diphtheriæ also give different results. Thus some will grow much more rapidly and vigorously on blood serum than others. In the vigorous cultures the different stages of development are passed through more quickly than in the weaker growing forms. In some strains large granules develop in short, even bacilli with round ends and have little tendency to grow out into the long, clubbed forms. Others (like the one which was photographed for the plates) have small granules develop in bacilli with faint staining pointed ends and later develop into long, clubbed forms.

Branching was not observed in any of the ten strains studied in this work. At all times when this has been observed by the writer there have been long, clubbed, and granular forms present.

To recapitulate: In the growth of the diphtheria bacillus on serum, we find during the first eight to twelve hours solid staining bacilli frequently arranged in pairs. The growth during this time is characteristic of all bacilli and consists in elongation of the rods and binary fission.

With the appearance of growth on the serum there begins a series of changes in the individual bacilli. They elongate without fission, metachromatic polar granules appear, one end of the rod may become swollen, the protoplasm may break up into segments, long filaments may be formed, and rarely also true branching occurs. All these changes are characteristic of the higher bacteria and especially of the streptothrix.

From the above it is seen that the development of the diphtheria bacillus may be divided into distinct stages. In the first stage we have the organism multiplying, as all bacilli do, by elongation and fission, and to this might be given the name "*solid staining*," or "*bacillary stage*." In the second we have a "*higher stage*" of development where changes take place in the individual organisms similar to those seen in the streptothrix.

The changes which take place in this latter stage have been and are still held by many to be the result of degeneration. The metachromatic granules are considered by A.[18] and M.[8] Neisser and many others to be degenerative changes. The long, clubbed, and filamentous forms of the bacilli are commonly spoken of as "degenerative" forms.

Against these changes being the result of degeneration are the following reasons: (1) These changes may begin after less than eight hours of growth, at a time when degenerative changes would not be looked for. (2) They are not characteristic of degeneration seen in other bacteria. (3) These changes are associated with marked growth of the individual organisms. (4) They are like the changes seen in the developmental stage of the higher bacteria.

The conditions which bring about the transition from the solid staining or bacillary stage to that of the higher development appear to be dependent upon the accumulation of the bacilli or the products of their growth upon the serum. The transition usually begins with the appearance of growth on the surface. It appears earlier if the colonies are near together. The conditions for the transition appear to be somewhat analogous to those of spore formation.

EFFECT ON THE DEVELOPMENT OF B. DIPHTHERIÆ OF CERTAIN VARIATIONS IN CONDITIONS OF GROWTH. — VARIATIONS IN TEMPERATURE. — *Growth at room temperature 19°–21° C.* At the end of twenty-four hours there is, as a rule, no visible growth. The preparations show solid staining forms similar to four and eight hour growths in the incubator. At the end of forty-eight hours the growth is usually just visible and we find microscopically a few granular forms. In seventy-two hours the growth is usually abundant and the granular forms are well marked. The bacilli are short with round ends and large granules. As is always the case where bacilli of this kind predominate, there is less tendency for the bacilli to grow out into long or clubbed forms. The granules in bacilli grown at this temperature more often show metachromatism than when incubated.

Growth at 40° C. — This also has the effect of delaying the appearance of granules, and macroscopically growth appears somewhat later. Fifteen and twenty-four hour cultures may show only large, thick, solid staining forms. Neisser[8] has called attention to the fact that in cultures grown at a temperature above 36° C. the granules may not be shown with his stain.

REACTION OF MEDIA. — *Acid serum* (+ *two per cent*). — Serum with a reaction of + two per cent and upwards delays the growth of the bacilli. Only solid staining forms may be found in twenty-four-hour cultures. After forty-eight hours the bacilli are short and sometimes oval in shape with well-marked granules.

Alkaline serum (— *two per cent*). — The effect of this is less marked, although the solid staining stage is usually somewhat prolonged. In some cultures there seems to be more tendency for the bacilli to show barred forms than on ordinary serum.

SYMBIOSIS. — The changes which have been described take place in pure cultures. The diagnosis of diphtheria is made with mixed cultures from the throat, hence the importance of studying the effects of symbiosis.

Growth with staphylococci. — The staphylococci are the most common forms found in throat cultures. Staphylococcus pyogenes aureus was used in combination with each of the ten diphtheria cultures. An emulsion in distilled water was made of the staphylococcus and then mixed with the emulsions of B. diphtheriæ in varying proportions. From these mixed emulsions serum tubes were inoculated, and preparations were made at intervals.

It was found that there are difficulties in getting bacteria to grow together in a uniform way, and especially to regulate the relative amount of growth of each. In cultures, however, which showed satisfactory growths of each organism, certain effects obtained were quite constant. The effects produced were in part dependent on the relative number of cocci and diphtheria bacilli. If there were only a few cocci, the growth of the bacilli seemed to be rapid, and granular forms appeared in eight hours, even earlier than in pure cultures. If the relative number of cocci were very large, the granular forms were not found so early, often appearing later than in pure cultures. In rare instances, fifteen-hour cultures in which there were only a few bacilli and many cocci showed only a few solid staining forms.

Growth with streptococci. — The growth of the streptococcus during the first twelve hours is very rapid, but soon ceases. A few streptococci growing with B. diphtheriæ hasten the appearance of granules very much. Relatively large numbers of streptococci were never observed to retard their development.

The granular forms in mixed cultures are typical even forms with round ends and large granules (C and D). There is less tendency for the growth of long, clubbed forms than in pure cultures.

To recapitulate: The variations in the conditions of growth which have been studied — high and lower temperature, acid and alkaline media, and symbiosis — have all, with one exception, resulted in delaying the development of the bacilli and in prolonging the solid staining stage. The exception is the growth of B. diphtheriæ with a *few* staphylococci or streptococci, under which conditions these granular forms may appear even earlier than in pure cultures. All of these changed conditions, except perhaps the alkaline media, have the effect of producing short, even forms with large granules and with less tendency to growth into the long, clubbed forms.

Only a few of the variations in the conditions of growth to which cultures may be subjected have been studied. It seems probable that anything which makes the environment less favorable would prolong the solid staining stage and make an early diagnosis more difficult. This shows the importance of accuracy of technique in diagnostic work.

The observations which have been made of the effect of symbiosis on the morphology of B. diphtheriæ seem to throw some light on the observation already referred to, that during convalescence there is a tendency for the solid staining forms to replace the granular forms. At the time when cultures are made for diagnosis, and also when the first cultures for release are made, there are relatively many diphtheria bacilli and few of the other throat bacteria. The conditions are thus the most favorable for the early and characteristic development of granular forms. As convalescence advances, the diphtheria bacilli become fewer, and the throat bacteria whose growth had perhaps been somewhat checked during the disease now become more abundant. We thus have the conditions for a later development of granular forms, and in the fifteen-hour cultures for diagnosis the solid forms will be more abundant.

BACILLUS PSEUDO-DIPHTHERIÆ. — Thirty-five cultures of this bacillus have been isolated by the writer, — three from convalescents from diphtheria, four from cases of tonsilitis, and twenty-eight from healthy persons. All have corresponded to the descriptions of the bacillus usually given. All have produced alkali in sugar bouillon except three which did not affect the reaction of the media at all. All have caused clouding of the bouillon. None of them were virulent to guinea pigs.

The cultures of all ages have shown solid staining forms (C^2 and D^2). The bacilli are very similar to the young solid staining diphtheria bacilli, but as a rule they are shorter, thicker, and have more rounded ends. When individuals are found isolated as in mixed cultures they are absolutely indistinguishable from the young diphtheria bacilli.

Three of the cultures which corresponded in every other respect showed occasional granules. The granules were very small and were present in small bacilli with slightly pointed ends, not at all characteristic of B. diphtheria. A few of these granules were stained by Neisser's method. They were very small, however, and should not lead to errors in diagnosis, as no similar granular types have been met with in any cultures of B. diphtheriæ. The occurrence of these small granules in B. pseudo-diphtheriæ have been mentioned by Kurth,[11] Cobbett,[19] Gramakowsky,[20] and others, and Neisser himself emphasizes the fact that bacilli containing granules must be typical in every respect in order to be considered diphtheria bacilli.

The most marked and distinguishing characteristic of the B. pseudo-diphtheriæ is that instead of growth into long forms in the older cultures, the bacilli *are actually shorter* in twenty-four and forty-eight hour cultures than they are at the age of twelve hours. This condition, which has been found constant in all cultures, is well shown in the photographic plates. (Figs. 11 and 12.)

Six cultures of B. pseudo-diphtheriæ have been studied in serial preparations and under varying conditions as was done with B. diphtheriæ. This has shown that B. pseudo-diphtheriæ presents no marked changes in its morphology at different ages and under varying conditions. In old cultures grown at room temperature for a week or more some thick, swollen forms may be seen. These, with the appearance of granules in the three strains already referred to, are the only indication of a higher stage of development in this species. At all other times the bacilli were short, solid staining forms. No long forms and no branching were observed.

It is thus seen that B. pseudo-diphtheriæ has a development like young B. diphtheriæ, but no higher development like that of older B. diphtheriæ and streptothrix.

Bacillus pseudo-diphtheriæ is present in the nose and throat of over twenty-five per cent of all healthy persons.[18] It is possible, as has been suggested, that they become even more abundant during convalescence. This would further explain the increased frequency of solid staining forms during convalescence.

As B. pseudo-diphtheriæ and B. diphtheriæ are indistinguishable when met with in mixed cultures it may be impossible to make an immediate diagnosis of a fifteen-hour culture in which only solid forms are found. In the writer's experience, *longer incubation* will always settle the question. If the bacilli are true diphtheria, granular forms will appear in twenty-four hours. If no granules are seen it will be found that the bacilli when isolated have all the characteristics of B. pseudo-diphtheriæ.

If there are many solid forms in a fifteen-hour culture and no granular forms, it is probable that they are the pseudo-diphtheria bacilli, because, as we have seen, where there are many B. diphtheriæ in mixed cultures the granules appear early. If only a few solid staining bacilli are present they may be true diphtheria bacilli in which, as frequently happens under such circumstances, the granules have appeared late. Such a culture should be reported as doubtful and incubated longer.

BACILLUS XEROSIS. — Several varieties of bacilli which resemble the diphtheria bacilli have been found in the eyes of healthy persons. The one studied has resembled the diphtheria bacilli the most, and has corresponded with the description of B. xerosis given by Migula.[21]

Eight cultures have been studied in the same way as the B. diphtheriæ. All have produced acid in dextrose bouillon, though as a rule less than the B. diphtheriæ. None of them have shown virulence for guinea pigs.*

Eight and twelve hour cultures show solid staining bacilli very similar to B. diphtheriæ and B. pseudo-diphtheriæ, although usually somewhat smaller.

Fifteen-hour cultures show slightly longer rods. In addition to the single break in the protoplasm seen in the younger forms a suggestion of a light staining area in either half may appear, which is the first indication of segmentation. Very rarely short granular forms are seen.

Twenty-four-hour cultures. — The bacilli are longer. The barred or segmented appearance is more marked. A few granular forms may be seen, but they are not usually characteristic.

Thirty-six-hour cultures. — The bacilli may be longer and

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* None of these eight cultures of B. xerosis nor any of the thirty-five cultures of B. pseudo-diphtheriæ have shown any local lesions in guinea pigs at the point of inoculation. The method of differentiation advocated by Spronck would thus have been entirely inapplicable in the case of these cultures.

show more marked segmentation depending upon the amount of development in twenty-four hours.

Forty-eight-hour cultures. — A few forms may have begun to take the stain faintly and are breaking up. In these older bacilli certain of the segments seem to retain their staining properties longer than others, just as in a chain of streptococci certain individuals appear to retain their vitality longer than others (arthrospores?). A similar observation has been made in the case of barred forms of B. diphtheriæ.

VARIATIONS IN THE CONDITIONS OF GROWTH. — At room temperature there is no growth of B. xerosis if recently isolated.

Acid serum has the effect of delaying the growth and of causing granular instead of barred forms to appear. The bacilli are short and thick and may have good sized granules.

Symbiosis. — The effect of the growth in mixed cultures with staphylococci and streptococci is to increase the number of granular forms in fifteen and twenty-four hour cultures. No barred forms at all may be present, and the bacilli may resemble very much B. diphtheriæ. As a rule, however, the granular forms of B. xerosis are thicker and shorter and the granules are less sharp. An important means of distinguishing them is by Neisser's stain, by which only an occasional granule will be stained, while in cultures of B. diphtheriæ showing granular forms with Loeffler's blue a larger proportion of the bacilli will show granules by Neisser's method.

To recapitulate: Bacillus xerosis in young cultures show solid staining bacilli like young B. diphtheriæ and B. pseudodiphtheriæ. Like B. diphtheriæ, it has a higher stage of development characterized by elongation of the rods and the breaking up of the protoplasm into segments. Growth on acid media and symbiosis with staphylococci and streptococci causes a development of granular forms which are very similar to B. diphtheriæ.

The close resemblance of B. xerosis in mixed cultures to B. diphtheriæ is of practical importance, inasmuch as these bacilli may pass through the lachrymal duct into the nose and throat and lead to errors in diagnosis. The writer has five times isolated B. xerosis from the nose and throat of healthy persons. It has also been noted that when found in the nose or throat a culture made on the following day from the same person will usually fail to show their presence. This is what would be expected where the bacilli were not vegetating on the mucous membrane where found, but had come there, as it were, by chance. The presence of B. xerosis in a culture for diagnosis should always be suspected when granular forms are found which are not perfectly characteristic and which do not stain well by Neisser's method.

RELATION OF THESE THREE SPECIES. — In young cultures all three are similar. All are solid staining rods, frequently paired, parallel to each other, and, according to H. W. Hill,* all show the characteristic post fission movements.[22] In older cultures differences are seen. B. pseudo-diphtheriæ has no development like the higher bacteria. B. xerosis has a higher development characterized by the elongation of the rods and the breaking up of the protoplasm into segments. B. diphtheriæ has a more marked development as a higher form, showing clubbed, barred, and filamentous forms, and occasionally true branching. These observations suggest a close relationship and common origin of the three species. It may be that B. diphtheriæ and B. xerosis have developed by evolution from simple forms like B. pseudo-diphtheriæ, each passing through, in its early life, a stage of development corresponding to the early development of the race; or it may be that the B. pseudo-diphtheriæ represents a reversion from a higher type.

This work has shown that these three species are indistinguishable morphologically at certain stages and under certain conditions. All of them, however, in the cultures which have been studied, have shown characteristics sufficient to separate them when all stages of their development have been observed.

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* Dr. Hill has very kindly examined three each of my cultures of B. pseudo-diphtheriæ and B. xerosis by his "hanging block" method. He reports that both show the characteristic post fission movements ("snapping") which he has described in B. diphtheriæ. See "Journal of Medical Research," Vol. VII., p. 115 (1902).

TECHNIQUE. — All the tubes were inoculated with one or two loops of an emulsion in sterile water of a twenty-four-hour growth on serum. The preparations were made with a drop of distilled water on cover glasses or directly on the slides, dried in the air, fixed with very gentle heat, and stained with Loeffler's methylene blue (Grübler) one to three minutes without heat.

Acid and alkali production. — Tubes containing just five cubic centimeters of a one per cent dextrose bouillon (reaction + 1 per cent) were inoculated with the bacteria to be tested; incubated at 36° C. for two days; titrated with $\frac{n}{20}$ NaOH and the reaction compared with that of the controls.

Tests of virulence. — Bouillon cultures grown for one week, slanted at 36° C., were inoculated subcutaneously to the amount of at least 0.5 per cent of body weight of guinea pig. The amount of the cultures of B. pseudo-diphtheriæ and B. xerosis inoculated was often more than 1 per cent of body weight. In the earlier inoculations plain bouillon was used; later, sugar free bouillon (Smith) with reaction of + 1 per cent was employed.

(I wish to express my thanks to Messrs. J. A. C. Nyhen, W. R. McAusland, and C. L. Overlander for help in making the cultures and preparations.)

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DESCRIPTION OF PLATES.

PHOTO-MICROGRAPHS BY MR. LOUIS BROWN.

The magnification is the same in all — x 2000.

All of the preparations were made from growth on blood serum.

PLATE VII.

Fig. 1.— B. diphtheriæ, 5 hours at 36° C. This shows only solid staining forms.

Fig. 2.— B. diphtheriæ, same culture, 8 hours at 36° C. This also shows solid forms, many of them with parallel arrangement.

Fig. 3.— B. diphtheriæ, same culture, 12 hours at 36° C. The bacilli stain faintly at their ends, and in some small granules are seen at the tip of the faintly stained portions.

Fig. 4.— B. diphtheriæ, same culture, 15 hours at 36° C. The bacilli stain more unevenly, and the granules are larger.

Fig. 5.— B. diphtheriæ, same culture, 24 hours at 36° C. This shows clubbed and barred forms as well as granular forms. At the lower part of the field is a paired form which shows the characteristic clubbing of the distal ends.

Fig. 6.— B. diphtheriæ, 48 hours at 36° C. This is the same bacillus as in the preceding figures, but from a culture where the colonies were discrete. It shows long filamentous forms.

PLATE VIII.

This is the same bacillus as in plate VII.

Fig. 7.— B. diphtheriæ, 24 hours at room temperature (20° C.). This shows only solid forms.

Fig. 8.— B. diphtheriæ, same culture after three days at room temperature (20° C.). This shows short forms with large granules. No long, clubbed forms are found.

Fig. 9. — B. diphtheriæ, in mixed culture with a *few* staphylococci, 15 hours at 36° C. The granules appear earlier, and are larger and more marked than in most pure cultures of this age.

Fig. 10.— B. diphtheriæ, in mixed culture with *many* staphylococci, 15 hours at 36° C. Most fields of the preparation from which this plate was made showed only staphylococci. Where there are relatively few bacilli, as here, the solid staining stage is prolonged and the granules appear late. In contrast to Fig. 9, this shows only solid forms, although of the same age.

PLATE IX.

Fig. 11.— B. pseudo-diphtheriæ, 11 hours at 36° C. This shows solid forms like the young B. diphtheriæ, but shorter, thicker, and with more rounded ends.

Fig. 12.— B. pseudo-diphtheriæ, same culture after 24 hours. Comparison with Fig. 11 shows the diminution in size in the older cultures.

Fig. 13.— B. xerosis, 12 hours at 36° C. This shows only solid forms.

Fig. 14. — B. xerosis, same culture after 24 hours at 36° C. This shows the characteristic segmented or barred forms which are characteristic of pure cultures of this species.

Fig. 15.— B. xerosis, in mixed culture with streptococci, 19 hours at 36° C. This shows the effect of symbiosis on the morphology. Granular forms are present which very much resemble B. diphtheriæ.

A NEW TECHNIQUE IN METABOLISM EXPERIMENTS.[1]

FRANCIS H. MCCRUDDEN.

(From the Laboratory of Physiological Chemistry at the Massachusetts General Hospital.)

During the past two years I have been carrying on a series of metabolism experiments at the Clinical Laboratory of the Massachusetts General Hospital in pursuance of an investigation started by Drs. Goldthwait, Painter, and Osgood upon the pathology and chemistry of certain chronic bone and joint diseases. Their purpose was to determine, if possible, what departure from normal metabolism occurs in rheumatoid arthritis, meaning by this the disease characterized clinically by atrophy of bones and cartilages, pain, swelling, and loss of function in various joints, and more or less chronic, constitutional disturbance. In addition to six or seven such experiments, two or three have been carried through in osteo arthritis and one upon a case of Paget's Disease of Bone (Osteitis Deformans). One experiment among the rheumatoid set has been repeated upon the same patient at an interval of one and one-half years, during which time all acute manifestations in the joints have given place to chronic conditions only.

These experiments were undertaken because of their belief that the morbid anatomy of this disease is not explicable on the grounds of any recognized pathological process and in the hope that physiological chemistry might be able to throw some light on the matter.

This first preliminary report, for it is to be regarded in no other light, will set forth the details of the technique pursued, and make mention of new methods of investigation which have been found to be exact as well as to point out defects used by other experimenters which have been shown to be open to criticism.

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Such complete metabolism work has not been done before in the investigation of this disease.

The study of metabolism, especially the metabolism of the inorganic constituents of the food, has given very interesting results even in the little time we have devoted to it, and the development of a practical method of carrying out a normal metabolism experiment has opened a rich field of great scientific interest. I refer to the study of the metabolism of the inorganic elements, not only in bone diseases, but also in other diseases and in health, for practically nothing is known of the subject in either condition. This ignorance undoubtedly comes from the fact that the determinations of the inorganic elements — phosphorus, calcium, magnesium, and sulphur — required by a metabolism experiment of a week or more, carried out under former methods would take a great many months to complete, even though the diet were considerably restricted.

A number of men have studied the relation of the calcium content of the food to rickets, and the results are rather contradictory. Bunge in his widely read book on physiological and pathological chemistry has plainly stated that all speculation on the theory of this subject is quite useless until we have careful and reliable experiments on the metabolism of rickety children, compared with that of healthy ones of the same age and brought up on the same food. He suggests, likewise, a study of the metabolism in osteomalacia. Yet neither he nor any one who has read his book has undertaken this work, probably on account of the time which would have been necessary for the analyses.

The method formerly used in making metabolism experiments has been, in brief, to feed a patient on weighed amounts of certain foodstuffs, and then by a determination of the per cent of nitrogen and other constituents to be studied in each article of food given, to find the total amounts of nitrogen and other elements administered. These elements are then determined in the feces and urine, and the amounts ingested compared with those excreted. In order to cut down the time required for the analysis of the foods, the diet has always been considerably restricted. The patient is given the necessary amounts of proteid, fats and carbohydrates, without any special attention to the palatability of the foods.

This restriction of the diet certainly introduces an undesirable element into the experiment, since a qualitative or a quantitative change in the diet very often produces changes in the metabolism of certain elements and compounds. We know that animals can live on milk alone, yet Lunen[1] has shown that if casein, fat, carbohydrates, and inorganic salts are mixed in the same proportions as they are found in milk such a mixture will not support life very long. We know that experiments tried on soldiers to determine the efficiency of condensed foods show that although the food is scientifically correct in the number of calories given, and in the weights of proteid, fats, and carbohydrates, the soldiers cannot do their work after a week or so of such diet.

A Vienna chemist carried out a metabolism experiment on a patient who had arthritis deformans in which he studied the metabolism of calcium, magnesium, and phosphorus. The patient was fed for a week on thin soup and milk. This chemist wrote a long article and drew some very explicit conclusions from this one nearly worthless experiment. In order to get valuable results we must do a large number of experiments, and give the patient a mixed diet as nearly as possible that to which he has been accustomed.

In order to decrease still more the number of analyses it has been customary to keep one article of food several days. One loaf of bread or a quantity of milk, for example, is fed to a patient as long as it lasts, and only one analysis of each made. As it is almost impossible to keep the moisture content of food the same over a period of a few days, or even a few hours, this keeping of the food introduces inaccuracy into the work. Bread, for example, will become dry and the percentage of proteids and other solids will therefore increase.

The restriction and change of diet and the staleness of the food, due to keeping it several days, may decrease the patient's appetite and make him depressed. This again is undesirable, for patients undergoing a metabolism experiment are likely to become peevish, especially toward the end, and their mental condition may indirectly influence their metabolism.

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[1] Zeit. fur Physiol., V., 311, 881.

In my method of performing these metabolism experiments all objectionable features of the old methods, such as the time required for many analyses, the inaccuracy of analyses due to keeping the food, the undesirability of restricting or changing the diet, and the staleness of the food due to keeping, are entirely eliminated. The foods taken for analysis are all mixed altogether in the same proportions that they are given the patient, and the mixture analyzed as a whole. This makes but one analysis of the food necessary, and permits giving the patient an unrestricted diet. The food given is always fresh and the inaccuracy due to keeping overcome by weighing the patient's food and that for analysis at the same time.

In feeding to the patient a liquid food, it is mixed as thoroughly as possible, a certain volume given him, and the same volume taken for analysis. A solid food is mixed after being cut into small pieces. The patient receives a certain weight of it and the same weight is taken for analysis. The mixing and weighing is of course done at each meal during the experiment. The sugar and salt used can be determined by weighing out a quantity of each at the beginning of the experiment and determining what is left at the end. Only non-homogeneous foods are excluded by this method. In weighing a piece of mince pie, for example, we could not tell how much was crust and how much was mince meat.

At the end of the experiment all the food taken for analysis is well mixed together, and after addition of a little hydrochloric acid to retain nitrogen, as much water as possible is evaporated on the steam bath. The rest of the moisture retained by the food is carried off by mixing the food with alcohol and evaporating a couple of times. The food is put through a grinder and afterwards the fat is extracted with naphtha. It is more easily ground up finely when free from fat. Finally, after the food is crushed so that it will all pass through a fine meshed sieve, it is sampled by quartering.

In quartering, the food is thoroughly mixed with a large spatula and then made into a little circular pile a couple of inches high. The pile is divided into four nearly equal quarters. Two opposite quarters are rejected, and the other two well mixed, made into another little pile, and quartered again. The mixing and quartering are repeated until there is about enough food left for one set of analyses. Samples for duplicate and triplicate analysis are obtained in the same way.

The following results show that the mixing can be very complete. The samples "A," "B," and "C" represented as nearly as possible entirely different portions of the food:

	CaO	MgO	P_2O_5	cc. of — H_2SO_4 used by the N in the Kjeldahl method.
"A"	.1026 grams	.1128 grams	.1672 grams	20.41 cc.
"B"	.1012 "	.1112 "	.1680 "	20.64 cc.
"C"	.1016 "	.1120 "	.1659 "	20.46 cc.

That in the extraction of fat by naphtha we do not extract any calcium, magnesium, sulphur, or nitrogen, is proved by the following analyses "D" and "E," which are the results of the analysis of two samples of dry food. "F" is the analysis of the dry food to which a corresponding part of the naphtha solution has been added.

	CaO	MgO	S	H_2SO_4 used by the N in Kjeldahl.
"D"	.1370 grams	.0852 grams	.1451 grams	39.62 cc.
"E"	.1356 "	.0858 "	.1480 "	39.76 cc.
"F"	.1354 "	.0848 "	.1452 "	39.68 cc.

I have not had occasion in my work to determine fats or carbohydrates, but this method can undoubtedly be used for the determination of these compounds; in fact, is now being tested to determine whether there are any sources of error in using it to study the metabolism of fat.

The experiment is started by starving the patient from noon of the day before the experiment until the next morning. This is done in order that the contents of the alimentary canal may pass along and not mix with the food given during the first part of the experiment. Half an hour before breakfast on the first day of the experiment the usual charcoal mixture is given.[1] This blackens the first stool belonging to the metabolism experiment. The urine is collected from eight each morning until eight the following morning. If the urine were collected from evening to evening considerable of the nitrogen, sulphur, and so forth, might come from the previous day's food. The separation of the different days' urine is desirable in order to study the daily variations in excretion. The urine each day is made up to a definite volume and aliquot parts are taken for analysis. In order to have as complete as possible a separation of urine from feces, the bladder is emptied just before stool. Washed filter paper as free as possible from impurities is used after stool and added to the feces. The same weight of filter paper is added to the food used for analysis in order that the impurities in the paper mixed with feces will be offset. The feces are dried, ground up, and well mixed for analysis. If a patient vomits, the vomitus should be analyzed separately and the results subtracted from the food.

The experiment is ended by starving the patient from noon on the last day of the experiment until the following morning. Half an hour before the first breakfast after the experiment is over the charcoal mixture is given. The feces are saved until they become black.

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[1] 10 gms. charcoal, 10 gms. acuim, 60 cc. peppermint water, well shaken together.

AN ENCAPSULATED DIPLOCOCCUS IN MASTOIDITIS.[1]

MARY S. PACKARD, M.D.

(*From the Pathological Laboratory of the Rhode Island Hospital.*)

When in the June number, 1901, of the Journal of the Boston Society of Medical Sciences (Vol. V., p. 499) Dr. Oscar Richardson described an organism found four times in the lung at autopsy, which he named the Pseudo-Pneumococcus, a diplococcus was being studied at the Rhode Island Hospital resembling the pneumococcus, but not fully identified with it, obtained from the mastoid at operation. The description of the pseudo-pneumococcus showed an organism which had the same points of difference from the diplococcus pneumoniæ, as did the one from the mastoid. In April, 1902, a second diplococcus with the same peculiarities was obtained from pus in the mastoid process, and in October, 1902, a similar organism, not in pure culture, was isolated from pus in the mastoid bone.

In view of the discussion brought up later as to the relation of capsulated streptococci and diplococci to the pneumococcus in the report by Howard and Perkins and one by Longcope, both in the Journal of Medical Research (Vol. VI., p. 163, 1901, and Vol. VII., p. 220, 1902), it seems worth while to describe a similar organism found three times in cases of mastoiditis, in two of the cases in pure culture. All three patients were in the service of Dr. Sprague at the Rhode Island Hospital, who kindly permits the use of a brief abstract of the clinical notes.

J. S., age 40; admitted to Dr. Sprague's service June 17, 1901. Patient complained of pain about the right ear, with some purulent discharge; there was some redness and swelling around the right ear. The duration of the trouble had been about three weeks. On June 18 the mastoid bone was opened and in the culture taken then the organism in question alone was found. July 5 the patient was discharged cured.

M. C., age 34, admitted April 25, 1902. History of the patient's trouble is of four months' duration, beginning with a sensation of fulness in the

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[1] Received for publication Jan. 29, 1903.

right ear, followed by a watery discharge which later became thick and yellowish and persisted till admission. One month before admission the patient had pain and swelling in the mastoid region which later spread to the face. A few days before admission an abscess behind the ear was opened and considerable pus escaped. At operation a cavity containing puriform material was found in the mastoid process. The capsulated organism in pure culture was obtained from this abscess, although the culture was not taken till the time of dressing the wound, four days after the operation. The patient was discharged May 22 cured. His temperature while in the hospital was practically normal except on the day after operation, when it reached 100°.

L. F., age 23, admitted Oct. 1, 1902. The history is of five weeks' duration. The trouble began with neuralgic pain in the left side of the head, sudden in onset, soon replaced by buzzing in the left ear, which persisted. Twelve days before admission there was slight swelling near the left ear, followed by tenderness over the left mastoid, which gradually diminished. At the operation on October 13 considerable pus was found in the mastoid cells and antrum. Patient was discharged cured October 31. His temperature, except on the day of admission, was normal.

In a culture taken from the discharge from the ear of the last case, nine days before the operation, the capsulated diplococcus was found, with the staphylococcus pyogenes aureus and albus. The culture taken from the mastoid at operation showed the same capsulated coccus and an unidentified bacillus which decolorized by Gram's method, presumably a contamination.

The coccus found in the third case differed from the other two in preserving, in smears, an arrangement in long, straight chains, usually of pairs. As it was less completely studied as regards its cultural properties, the peculiarities of only the first two will be noted in detail. Organism Number I. will be described, and the points of difference from it in Number II. mentioned.

Stained smears from the material swabbed out of the bone at operation showed a few pus cells and round cells, and a few cocci in pairs, retaining the stain by Gram's method.

Blood Serum. — Cultures from the same material showed no growth till the third day, when the fluid of condensation was clouded, and on the solid portion were scattered colonies the color of the medium, flat, irregular in shape, one to two millimeters in diameter, resembling flecks of oil. Anaerobically there was growth in the fluid only. In Number II. the first growth on blood serum was a confluent mass with oily appearance, but in later generations it grew in smaller masses and discrete colonies.

Litmus Milk. — Slight acidity in twenty-four hours, coagulation after three days; anaerobically, coagulation after two days. Number II. formed a little acid in aerobic growth and anaerobically caused coagulation in twenty-four hours. More rapid acid formation in anaerobic than in aerobic growth was further demonstrated by the reaction in milk in the fermentation tube. The organism from both cases caused coagulation and decolorization in the branch in from twenty-four to forty-eight hours, with only slight acidity in the bulb, the coagulum in the branch being sharply separated at the elbow from the fluid milk in the bulb.

Glucose Bouillon. — Diffuse cloudiness with acid formation in twenty-four hours both aerobically and anaerobically. In the fermentation tube there is clouding in the bulb and branch with acid formation in twenty-four hours; no gas. After two days a flocculent sediment is formed at the elbow. Number II. caused no clouding unless planted in larger than ordinary amounts of bouillon, about fifteen cubic centimeters. In the fermentation tube clouding spread gradually around the elbow and up the branch, requiring eighteen days to reach the middle of the branch. Number II. grew richly in the ordinary amount of glucose bouillon in a test tube, if to that were added the fluid of condensation of one blood serum tube, while in control tubes of the same bouillon in the same amount there was no growth.

Glucose-free Bouillon. — Clouding and acid formation in twenty-four hours; no indol. Anaerobically the clouding appeared later and was less. Number II. failed to grow in glucose-free bouillon, but plants in large amounts were not tried.

Glucose Agar. — Fluid of condensation clouded; fine white growth in line of stab. Colonies on slants and on Pétri plates are very small and colorless after twenty-four hours, growing in two days to a diameter of about one millimeter. They have the oily appearance of the colonies on blood serum, but are opaque at the borders. They grow paler and gradually more indistinct, and after a varying length of time, from four to twelve days, are not visible. In anaerobic tubes there are no surface colonies.

Potato. — Growth is invisible, but present.

Gelatin. — No visible growth aerobically nor anaerobically. Number II. produces a faint growth in the line of stab after three days; this increases in density without liquefaction.

Viability. — The organism dies rapidly in most of the artificial media, and while still pathogenic for mice is not capable of growth by transplantation on media. Number I. lived longest in the fluid of blood serum; Number II. was viable after thirty-six days in gelatin.

Morphology. — The most marked characteristic of the organism morphologically is the variety of forms which it takes on different media, after different periods of growth on the same medium, and in different generations of life on artificial media. Smears from a twenty-four-hour growth on blood serum in the first generation from the human body show cocci in pairs or chains of pairs, often lanceolate, the two units well separated or so closely united as to appear like a bacillus pointed at both ends. By Gram's method, the cocci are usually stained black or dark brown, surrounded by a light brown halo, indefinite in outline. Stained by Welch's method, the cocci are deeply stained, the capsule of about the same width as the diameter of the coccus, pale, with a deeply stained, distinct border. In some of the other media and after longer growth on blood serum, greater irregularity in form, grouping, and staining are shown. Empty capsules, forms decolorized by Gram's stain, or retaining a central dot of deeply staining substance are seen. Short chains made up of individuals capsulated and not capsulated, differing in size, faintly or deeply staining, are shown. Capsules by Gram's stain are not so frequent in these preparations, and often it is difficult to demonstrate them by Welch's method, in cultures after the first generation from the animal body. In general it may be said that in young cultures in the media in which growth is comparatively rich the morphology and staining properties of the organism resemble most those of the pneumococcus. It is in the old cultures in favorable media and in young cultures in media in which growth is slow and poor that the unusual forms and varieties in staining are seen. Chain formation is poor except in fluid media; it is good in hanging drop preparations. The organism is non-motile at room temperature and higher.

Subcutaneous inoculation of mice caused death, usually in less than twenty-four hours. In one case there were some subcutaneous edema, in the others no change at the point of inoculation and no macroscopic abnormality of the organs. The micro-organism was obtained in pure culture from the blood and organs, growing with the same cultural and morphological characters as when taken from the human body. In stained smears from the blood and organs of mice it appears as a symmetrical diplococcus, and would here be indistinguishable from the pneumococcus but for the demonstration of the capsule without the special capsule stain.

A STUDY OF THE NECROSES OCCURRING IN THE LIVERS OF EXPERIMENTAL ANIMALS AFTER INOCULATION WITH HOG-CHOLERA BACILLI.[1]

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In the livers of rabbits and mice that have died from the effects of inoculations of hog-cholera bacilli there occur small characteristic grayish or yellowish white patches involving one or more lobules. These patches are areas of coagulation necrosis, and while they vary considerably in number and size, they are of extremely constant occurrence in such animals. They attain, usually, their greatest size in the thin dependent borders of the liver. The superficial lobules are most often affected, yet at times the deeper ones are also involved.

These necroses were first described by Klein, who observed them in the livers of rabbits and mice inoculated either with tissue or cultures taken from hogs that had died of pneumo-enteritis or pig typhoid. He described both a bacillus and a micrococcus in the necrotic areas, but he was inclined to consider the bacillus the more important organism of the two.

Somewhat later Theobald Smith showed that these peculiar liver necroses constantly followed the inoculation of rabbits and mice with a fatal dose of his hog-cholera bacillus, provided the animals survived sufficiently long (seven or eight days).

Both Klein and Smith considered these patches as areas of coagulation necrosis due to a plugging of the vessels; but neither at the time was able to show how this plugging occurred, nor did they give any detailed account of the necroses.

About four years ago Dr. Smith, in looking through some

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sections of mouse liver, noticed in some of the vessels adjacent to the necrotic areas, thrombi of a peculiar nature. They were apparently balls of more or less fused and altered red blood corpuscles.

This led him to suggest to me that I make a study of the necrotic changes occurring in the livers of animals inoculated with hog-cholera bacilli.

MATERIAL AND TECHNIQUE. — In order to trace the origin and development of the lesions, a series of mice was inoculated and certain of them killed at intervals. Mice were selected for these experiments because of their marked susceptibility to the disease and the constancy with which the necroses appear in their livers. In this series only tame mice were used, but in the remainder of the work both wild and tame ones were used.

The culture employed was one of a bacillus isolated from an outbreak of hog-cholera by Dr. R. R. Dinwiddie in Arkansas. It was of the α type of Smith, which type he describes as the most virulent. Although it had been in the laboratory about two years, and had been passed through a series of guinea pigs, it was very virulent to rabbits, and quite so to mice.

The mice were inoculated with .02 cc. of a twenty-four-hour bouillon culture, so diluted with sterile salt solution that 0.1 cc. was injected. This amount had previously been determined as about as small a dose as could safely be relied upon to produce death.

TABLE I.

	Sex.	Weight.	Killed hrs. after injection.	Red thrombi.
Mouse 23	Male	18 grms.	8 hours	—
" 16	"	14 "	21 "	—
" 18	"	10 "	21 "	—
" 22	"	16 "	20 "	—
" 20	"	15 "	46 "	—
" 21	"	12 "	46 "	—
" 12	"	19 "	72 "	—
" 13	"	14 "	72 "	—
" 10	"	24 "	95 "	—
" 11	"	18 "	95 "	+
" 17	Female	15 "	119 "	+
" 19	"	17 "	119 "	—
" 14	"	23 "	143 "	—
" 15	"	24 "	143 "	—

Unfortunately the mice used in this series varied considerably in age and weight, and on this account, in resistance, hence the results were not so uniform as desirable. Nevertheless they served the purpose fairly well.

In addition, the lesions were studied in twelve other mice that had died from the effects of injections of varying amounts of the same, and three other cultures isolated from different epizoötics in swine and furnished by Dr. Smith.

TABLE II.

	Sex.	Weight.	Culture.	Dose	Died.
Mouse 1....	Wild male	Adult	Arkansas ∝	0.05 cc.	5½ days
" 2....	" female	"	" "	"	7½ "
" 3....	Tame male	16 grms.	" "	0.025 cc.	7½ "
" 4....	" "	16 "	" "	"	5½ "
" 5....	" "	20 "	" "	"	5½ "
" 6....	" "	17 "	" "	0.0125 cc.	12[2] "
" 7....	" "	22 "	" "	"	7½ "
" 8....	" "	20 "	" "	0.025 cc.	7½ "
" 26....	Wild female	Adult	Massachusetts ∝	0.05 cc.	6½ "
" 27....	" "	"	Maryland ∝	"	7 "
" 31....	Wild	"	Non-motile[1]	0.30 cc.	6½ "
" 32....	"	"	"	0.12 cc.	8½ "
" 33....	"	"	"	0.06 cc.	5½ "

[1] Centralblatt f. Bakt., 1889, Bd. xxv, s. 241.
[2] Chloroformed.

Several other uninoculated mice, both wild and tame, were examined as controls.

From some experiments going on in this laboratory with this same disease I was able to obtain considerable rabbit and guinea-pig material. As visible liver necroses occur but rarely in guinea-pigs, little attention was given them, but so far as I have examined such as did appear, they resembled in general those found in mice and rabbits. The rabbit material was composed wholly of tissue from rabbits that had succumbed to the disease. The time they survived after inoculation varied from five and one-half to eleven and one-half days. While the necrotic processes taking place in rabbits are essentially the same as those in mice, they are not so easily followed, and for this reason the description following will apply for the greater part to mice, the chief points of difference being noted.

Tissue from chloroformed animals was fixed at once. But as those that were allowed to die of the disease almost invariably died during the night, the tissue from them was not so fresh, yet no appreciable difference could be seen.

The fixers tried were Zenker's, Flemming's, formalin, absolute alcohol, and acetic-sublimate. For general purposes Zenker's was the most useful.

Eosin followed by Unna's polychrom-methylene blue gave the most satisfactory stain. Delafield's hematoxylin with Van Gieson's picro-acid fuchsin, Ehrlich's triacid, safranin, and Weigert's fibrin stain with alum carmine were also used with good results.

The sections studied varied from two to six microns in thickness.

PHAGOCYTIC-CELL THROMBI. — On examining sections of the liver of a mouse killed twenty-four hours after an injection of hog-cholera bacilli, one notices clumps of large cells lying in the distended capillaries. These clumps are, perhaps, more numerous just beneath the capsule of the liver and the walls of the veins. The interlobular connective tissue, also, in certain regions is brought into prominence owing to the large number of these cells in its interstices.

The size of the cell foci varies much. They may consist of but three or four cells or of a considerable number. As a rule, however, they are, at this stage of the disease, yet of small size. The cells composing them may stain deeply with methylene blue in one clump, while in another neighboring one they may take a feeble eosin stain.

These cells are large mononuclear cells with a relatively large, generally eccentrically placed vesicular nucleus. The shape of the nucleus in the younger forms is oval or reniform. As the cell grows older, however, the nucleus becomes more irregular, assuming often a horseshoe or ring form (Fig. 1). The nucleus consists of a distinct, deeply-staining membrane with enclosed feebly-staining karyoplasm in which lie chromatin masses of varying size and shape. The cytoplasm of the newly formed cells stains deeply with most basic dyes, especially so with methylene blue. With polychrom-methylene blue it is metachromatic, assuming a violet tint. The cytoplasm in structure is finely reticular, resembling that of the large lymphocytes. Occasionally a few fine granules may be made out by certain staining methods.

These cells are of low vitality, and very soon begin to show degenerative changes. In cells with the horseshoe or ring-shaped nucleus, the enclosed portion of cytoplasm first shows this change. It takes a feeble eosin stain, while the remainder of the cytoplasm still stains with methylene blue. Later the whole of the cytoplasm is affected, and stains no longer with the basic dye, but takes a feeble eosin stain. (Fig. 2.) The degenerative changes in the nucleus are shown in its greater irregularity and the lessened intensity with which it takes the dye.

The form of cell usually met is round or elliptical, yet ameboid forms are not uncommon. That they are considerably larger than other blood cells, the following measurements from a blood smear fixed by heat will show:

Large mononuclear cells, 13.5 μ — 18.7 μ average diameter.
Polymorphonuclear leucocytes, 11.23 μ.
Small lymphocytes, 7.8 μ.
Red blood corpuscles, 6.59 μ.[1]

These large mononuclear cells possess phagocytic powers, and occasionally one finds within them red blood corpuscles, lymphocytes, and polymorphonuclear leucocytes. Bacteria have been observed in these phagocytes of the rabbits, but not in those of the mouse. The phagocytes of the rabbit are identical in structure with those of the mouse, except that in a considerable number of cells one sees many fine acidophile granules about the size of the finer eosinophile granules of the leucocytes.

One may now readily identify these cells with those phagocytic cells observed by Freidreich, Wagner, Billroth, Mallory, and others in typhoid. Flexner notes their presence in rabbits poisoned by abrin and ricin. Mallory pictures similar cells in the guinea pig. And more recently, under the title of "Endothelial Phagocytes of the Tonsillar Ring," Goodale describes cells of the same sort. The question immediately presents itself, Where do these cells originate?

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[1] Average diameter, according to Gulliver, being 6.7 μ.

In many mice the peritoneal endothelium of the surface of the liver shows marked signs of proliferation. Swelling of the nuclei and mitotic figures is common. Then again, the frequent presence of cell clumps near the surface of the liver argue in favor of the origin of a considerable number from this source. It is probable, also, that they arise from the endothelial cells of the lymph and blood-vessels of the interlobular connective tissue. In the rabbit they are plainly seen arising from the endothelial lining of both the hepatic and portal veins. In the mouse, however, this mode of formation is not so frequent, and it is safe to conclude that by far the greater number arise in the spleen and are carried into the capillaries of the liver. For on examining the spleen of an infected mouse, large masses of these cells in active division may be seen adhering to the muscular trabeculæ. These places of origin correspond with those given by Mallory for the phagocytes of typhoid. Recently Beattie has shown that cells of this sort arise from the endothelium of the omentum in guinea pigs. These cells are not confined to the portal system, but are found also in the general circulation, in the heart, the vessels of the kidney, adrenal, and skin, and cells that one cannot distinguish from them may be found in smears from a drop of blood from the tail. In fact, it is impossible to differentiate them from the large mononuclear leucocytes, with which, I believe, they are identical. Associated with these cells in the masses blocking the capillaries, we find desquamated endothelial cells, plasma cells, lymphocytes, and polymorphonuclear leucocytes, and less often, but not at all infrequently, eosinophile cells. These cell thrombi tend to increase in size by the addition of cells carried to them by the blood stream, and, to some extent, by multiplication of the cells composing them.

Having traced the history of the phagocytic cells we may now recognize as youngest those cell clumps in which the cells composing them stain with basic dyes (Fig. 1). With age the cells lose their power to stain with methylene blue and take a feeble eosin stain (Fig. 2). Farther signs of degeneration rapidly become apparent, the cells losing their outlines, their nuclei shrinking and fragmenting. Many show fatty degeneration.

Within forty-eight hours fibrin is deposited on and among the cells, binding them closely together. This fibrin deposition may take place throughout the cell clumps or be confined to but one side. As time passes, more fibrin is deposited, the cells disappear by liquefaction, and by the seventh day only a network of fibrin containing cell fragments remains (Fig. 3).

The formation of cell thrombi is not confined to the first few days, but takes place continuously up to the time of death. In animals that have been allowed to die, these clumps are found in all stages of formation and degeneration. In those mice that showed signs of recovery, invasion of these areas by polymorphonuclear leucocytes took place, but as none were allowed to fully recover, the ultimate fate of the lesions could not be determined.

The liver cells, as one would suppose, are not unaffected by this plugging of the capillaries by phagocytic cells, and they show marked necrotic changes. At the time of the first appearance of cell clumps, beyond a general swelling of the liver cells producing a narrowing of the capillaries, no other lesion is seen. Yet even before the phagocytes begin to show signs of degeneration, the adjacent liver cells begin to go to pieces. The nucleus fragments (karyorhexis), the cell wall nearest the thrombus, ruptures and the cell contents escape. Occasionally a somewhat different change occurs, — it is the more common in rabbits. The cytoplasm of the liver cells which normally in both animals stains a neutral tint, and contains basic staining particles on the side nearest the thrombus, shrinks, becomes hyalin, and takes a deep eosin stain. This change extends throughout the cell. The nucleus undergoes karyorhexis. These altered cells stain with Weigert's fibrin stain. The cell is crowded aside and the space it formerly occupied becomes filled with phagocytes. By the disappearance of several adjacent liver cells, the cell clumps may attain a considerable size and conform to the lymphoid nodules of the older writers on typhoid fever.

To decide definitely whether this disintegration of the liver cells is due primarily to the presence of the phagocytes obstructing the capillaries, or whether they may not be attracted by the disintegrating liver cells is difficult. There is some evidence in favor of the latter view, as isolated liver cells undergoing necrosis in the manner described may be found, in whose neighborhood no phagocytes are to be seen. Since the giant cells [1] of the bone-marrow, which are many times larger than the phagocytes, are often found passing through the liver capillaries without producing any necrosis, the idea that the large size of the phagocytic cells precludes their passing through, and thus produces an obstruction of the capillaries, cannot be maintained.

Owing to their obstruction, the capillaries, which at first are rather narrow, become over distended and packed full of closely approximated red blood corpuscles, which frequently are shadow forms. From the pressure so produced, the trabeculæ of liver cells are reduced in many lobules to narrow bands not more than half their normal width. They commonly show fatty and other degenerative changes. In rabbits, small masses of bile pigment are often found in them. The endothelium of the capillaries is separated in many places from the liver trabeculæ by a serous exudate. Hemorrhage not infrequently takes place into these spaces. When phagocytic cell thrombi occur in neighboring capillaries a number of liver cells may be surrounded and cut off by their confluence. These enclosed cells undergo coagulation necrosis.

In addition, a variety of changes, such as usually accompany disturbed metabolism, but which are not specific enough to warrant description here, take place in the liver cells. A more or less extensive fatty degeneration is frequently present.

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[1] Smith notes the normal occurrence of the giant cells of the bone-marrow in the spleen of mice. Jour. Exper. Med., Vol. VI., p. 1. In all the spleens examined I have found them in considerable numbers.

From some experiments of Mallory's it appears that in guinea pigs, at least, clumps of phagocytes are either normally present in the liver capillaries, or may be produced by rough handling. In order to determine if such were the case with mice, I examined sections of the livers of several mice, both wild and tame. One had had an injection of sterile bouillon. The others had not been inoculated and were handled as little as possible.

In every case such cell clumps were found. But they were smaller, and present in much smaller numbers than in the inoculated animals. The degenerative changes, too, in them were much less marked.

While the formation of phagocytic cell thrombi is by no means specific for this disease, it is plainly seen that they are greatly augmented in number and size by it, and the degeneration proceeds much farther than ordinarily.

In some mice clumps composed for the most part of cells considerably larger than those already described are found in the liver capillaries. Many of these cells are filled with an amorphous yellowish-brown pigment (hemosiderin?). One or more shrunken and degenerated nuclei may be present. Some of the cells stain with eosin such a way as to suggest that they may be phagocytic cells filled with partially digested red blood corpuscles. Smaller cells of the same sort are also found.

In the spleens of mice and guinea pigs the phagocytic cell clumps attain, in certain cases, a relatively large size. They undergo coagulation necrosis, and show on the surface of the organ as yellowish-white patches. In the spleens of rabbits they have not been observed, and if present are masked by the great congestion of that organ in these animals.

The lesions so far described are essentially the same as those found by Mallory in typhoid, and agree fairly well with those found by Flexner in rabbits poisoned by abrin and ricin. Reed, by the injection of typhoid cultures into the mesenteric vein of rabbits, produced in their livers apparently the same sort of lesions, but he gives to them a quite different interpretation.

HYALIN THROMBI. — In addition to the lesions given above, we have others of quite different origin, the yellowish-white patches first mentioned. While the first sort affect, usually, only a small portion of the liver lobule, the second involve the greater portion of one or several lobules (Fig. 4). The surface lobules are the ones most often affected, the reason for which we shall see when we come to discuss their origin.[1]

In sections stained with eosin-methylene blue the necrotic areas stand out in sharp contrast to the normal tissue (Fig. 4). The liver cells, while retaining their shape, stain deeply with eosin. The basic staining particles present in the normal cell are absent, and the cytoplasm shows a finely reticular structure instead of the normal granular one. In these cells the bladder-like nucleus of the normal cell has shrunken to less than half its former size. At first it stains rather darkly, but as the shrinking continues it loses its power to stain with basic dyes, and can be distinguished from the cytoplasm only by its greater density and hyalin appearance. Later it disappears completely.

The endothelium of the capillaries of these areas does not undergo degeneration so soon as the liver cells, and it remains unaffected for some time. The capillaries may be empty, but are generally filled with phagocytes and blood corpuscles. Invasion of these areas by polymorphonuclear leucocytes occurs but rarely — a fact noted by Smith. Phagocytic thrombi are often included in them.

In mice it is on the fourth day after inoculation that we first find these necrotic areas, and then, also, for the first time, one sees hyalin thrombi in the veins. On the fifth day both thrombi and anemic areas are more numerous.

The thrombi occur in both the portal and hepatic veins.

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[1] Dr. Smith tells me that foci of coagulation necrosis are most pronounced in the livers of rabbits inoculated with cultures recently isolated from swine. The power of the bacilli to produce these necroses appears to be lost, to a certain extent, by cultivation, and is not restored by passage through a series of rabbits. It is not possessed by varieties having a low degree of pathogenic power.

They may be free or attached to the vessel walls, and not infrequently they lie just beneath the endothelial lining which has been raised by a serous exudate. In size and shape they vary greatly. Those in the larger vessels have a spherical or ovoid form, while those in the smaller are cylindrical and often extend some distance along their axis. In the capillaries they are present as cylindrical plugs. They attain their greatest size in the hepatic veins and there they are found in greatest number, yet they are not uncommon in the portal veins.

These thrombi occlude the larger veins partially, but the smaller ones often completely. To this obstruction of the smaller veins, the portal especially, the necrotic areas already described are due, as one may readily demonstrate, by means of serial sections. That the surface lobules should be most often and extensively affected is to be expected, since their blood supply is less complete, and in case of the obstruction of one of the portal vessels fewer chances of establishing a collateral blood supply remain than in the case of the more deeply lying lobules.

When the obstruction occurs in the portal veins or the capillaries leading from them, the relation of the thrombi to the areas of anemic necrosis may be readily traced; but when thrombi are found obstructing the central veins of the affected lobules only, as appears to be the case in rabbits, it is more difficult to understand. In the latter case the portal veins leading to these areas are distended with blood, as are also the capillaries. The central vein beyond the thrombi is empty of red corpuscles. The necrosis is confined chiefly to the center of the lobule, the liver cells immediately adjoining the portal veins remaining unaffected.

In the capillaries also thrombi occur, and they, too, give rise to anemic areas which undergo coagulation necrosis. Owing to the hyalin structure of these capillary thrombi and their property of staining with Weigert's fibrin stain, it is difficult in many cases to distinguish them from fibrin masses or the hyalin-degenerated cells, yet they can generally be shown to be of the same composition as those present in the veins.

The thrombi (Fig. 5) are composed of a substance hyalin at the periphery and finely granular at the center. They are not homogeneous, but appear to be made up of a tangle of coarse cords among which a few phagocytes are caught. Fibrin threads cannot be demonstrated in them, yet may be deposited upon them and extend to the vessel walls. In the clefts at the periphery, normal blood corpuscles are often seen. Adhering to the thrombi are phagocytes, often in such numbers that they completely occlude the veins. The phagocytes are frequently bound together by fibrin.

These hyalin thrombi stain a violet-red color with eosin-methylene blue, and an orange or orange-red with picro-acid-fuchsin, in contrast to the lemon-yellow of the normal red blood corpuscles. With Weigert's fibrin stain they usually retain the stain, especially at the periphery. They also stain deeply with iron hematoxylin. In every case the periphery stains more deeply than the center. It appears that we have here to deal with the so-called hyalin thrombi.

Von Recklinghausen was the first to recognize them as of a class by themselves. He believed them to be derived from the leucocytes. Since then they have been described in many toxic states and infectious diseases by numerous writers. They have been found most frequently in the capillaries, in the form of hyalin casts. They have also been seen as balls and cylindrical masses in the smaller veins and arteries. Von Recklinghausen found them in spontaneous gangrene and ergotin poisoning, Kriege in the vessels of a rabbit's ear after freezing, Kaufman in corrosive sublimate poisoning, Ribbert observed them in rabbits poisoned with Staphylococcus pyogenes aureus, and Welch in the kidneys of cholera hogs. Mallory describes in the vessels of the intestine in typhoid fever what he calls " fibrin thrombi," but which are probably of this same nature. Klebs is of the opinion that these thrombi consist of coalesced and altered red-blood corpuscles. Ziegler states that they are formed from the colorless elements of the red blood corpuscles. Silberman, Werhofsky, and others also describe thrombi formed from more or less altered red corpuscles.

While the consensus of opinion appears to be that thrombi of this sort owe their origin to red blood corpuscles, nevertheless much doubt concerning their true nature still remains.

In sections of liver hardened in absolute alcohol and stained with methylene blue one sees the thrombi as brown masses of various sizes and of the same color as the red corpuscles. The brown color is due to hemoglobin and suggests at once what blood elements compose them. In most cases, however, the outlines of the red corpuscles cannot be distinguished, and from the structure of these alone one gets absolutely no clew of their origin. Yet by examining a large number one can trace the steps in their formation.

In what may be considered the first stage one sees groups of corpuscles lying more closely together than the remainder of the corpuscles in the same vessel. They also stain somewhat differently from the others. Next one sees clumps in which the corpuscles at the center are fused into a homogeneous mass, while those at the periphery retain their normal form to a certain extent. The corpuscles show a tendency to unite into cords which form a tangle in the larger thrombi. In the completely-formed thrombi the shape of the corpuscles can no longer be distinguished, and the center becomes finely granular.

Preliminary to and during the fusion of the corpuscles to form thrombi they suffer a change in their chemical composition, which is shown by their abnormal staining reaction. That the thrombi are not composed of shadow corpuscles, as Ziegler states, is shown by their brown color in unstained sections; yet, that the corpuscles composing them differ from the normal ones is shown by their tendency to retain basic dyes, such as methylene blue and gentian violet. In sections treated with Weigert's fibrin stain by which the thrombi are usually stained, we often find lying near them in the same vessel a few red corpuseles that also retain the stain, though the remainder are all decolorized. Hence the presence of fibrin is not necessary to account for this staining peculiarity.

The thrombi appear to acquire rapidly their final structure, for those found earliest in the disease are fully as far advanced as any found later. It is probable, although we find thrombi varying in size from those consisting of but a few corpuscles to those that would require many hundreds to give them their size, that most of them are of full size from the first and do not increase except by the adhesion of white cells to them and the deposition of fibrin upon them. Yet some of the larger appear to consist of several smaller ones more or less intimately united. Their formation does not occur wholly at one stage of the disease, but they continued to be formed from the fourth day after inoculation up to the time of death, immediately preceding which the process is probably most active, since we find at death thrombi in all stages of formation.

Stillmark has shown that ricin, when added in small quantities to defibrinated blood, causes an agglutination of the red corpuscles. Hellin has demonstrated a like action for abrin. Mitchell, Flexner, and others have made similar observations upon certain snake poisons. Experiments on the effects of introducing these poisons into the circulation have, however, not yielded such certain results. Still Werhovsky claims to have found thrombi formed by the fusion of red corpuscles in the capillaries of the liver of rabbits killed by abrin. Flexner failed to find any such thrombi in rabbits poisoned with abrin or ricin.

From the similarity of the action of ricin and abrin to many bacterial toxins, one could readily ascribe this clumping of the red corpuscles to form thrombi to the production by the bacteria of a toxin acting after the manner of the above poisons and producing agglutination of the corpuscles.[1]

Such a toxin might be produced directly by the bacillus and set free either during its life or upon its dissolution, or indirectly by the action of the toxin upon the tissues, causing such a substance to be liberated from them upon their destruction.

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[1] Shortly before the completion of this manuscript, Flexner presented before the American Association of Pathologists and Bacteriologists, at Cleveland, a preliminary communication "On thrombi composed of agglutinated red blood corpuscles." He has produced such thrombi by the injection of agglutinating substances — ricin, ether, globucidal sera. He has found them in cases of typhoid, bronchopneumonia, and carbolic acid poisoning.

The writer had no knowledge of this paper until it appeared in the November number of the Journal of Medical Research, 1902, Vol. viii, No. 2.

The latter method would also explain the formation of such thrombi in other instances, freezing, burning, and poisoning by certain mineral substances, in all of which cases a destruction of tissue takes place. That the bacteria play an important part in the formation of the thrombi is shown by their being formed in greatest numbers late in the disease when the bacteria are present in the largest numbers.

That any agglutinin is present, there is, as yet, no positive evidence and quite a different line of experiments would have to be undertaken to demonstrate its presence. I hope to follow this line up later. The idea is merely suggested here, as it seems to afford a satisfactory explanation of the phenomena.

RELATION OF THE BACTERIA TO THE LESIONS. — Search for the bacteria in the liver was made mostly in sections. The culture method was used only in the examination of the control mice for bacteria other than hog cholera which might give rise to phagocytic thrombi.

Material for sections was hardened both in absolute alcohol and Zenker's fluid. The sections were stained either with alkaline methylene blue and decolorized with dilute acetic acid or with eosin methylene blue. Both methods gave good results.

In none of the control mice were bacteria found by either method. In the section of the livers of the mice of the series killed at different intervals after inoculation, no bacteria which could certainly be identified as hog cholera bacilli were found on microscopic examination. Mice 11, 16, and 18 showed a few bacteria of doubtful identity, the remainder none.

Bacteria were found without exception in sections of the livers of all the mice that had been allowed to die of the disease. In most instances they were present in great numbers, but in others a few only were found after long and careful searching. In mouse 26, which was chloroformed while dying, bacteria were found in considerable numbers. The greatest number of bacteria was found in the livers of those mice which had lain longest before fixation. In these cases a post mortem multiplication probably took place. The bacteria were always found in clumps, some quite large, others very small. Bacteria were also constantly found in sections of the livers of rabbits which succumbed to injections of hog-cholera bacilli.

It is in the anemic areas that the bacteria are found in greatest numbers. They lie either in the capillaries or in the necrotic liver cells. They are present in the phagocytic cell thrombi and in capillaries surrounded by perfectly normal liver tissue.

The bacteria were quite often found in isolated necrotic liver cells. In many cases they appeared to be but the remains of bacteria, as they stain but feebly. Earlier in the disease, when these cells were first observed, no bacteria were found in them, so it is probable that the bacteria enter these cells only after the death of the latter, and are not the cause of their degeneration. In no other cases could any direct relation of the bacteria to the lesions present be demonstrated, and their connection here is exceedingly doubtful.

SUMMARY.

1. Two sorts of lesions are present. One is due to the plugging of the capillaries by large mononuclear cells, and probably, also, to the direct action of a toxin upon the liver cells. The other is due to the plugging of the smaller veins and capillaries by hyalin thrombi.

2. Early in the disease there is a marked proliferation of the endothelial cells of the blood and lymph vessels and serous surfaces. This proliferation is especially active in the lymph spaces of the spleen. The cells produced have but a low vitality, and possess, to a certain degree, phagocytic powers.

3. The hyalin thrombi are composed of slightly altered and coalesced red blood corpuscles.

4. No direct relation of the bacteria to the lesions could be shown.

(The writer wishes to express his thanks to Dr. Smith for the help and suggestions given him in this study.)

May, 1902.

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DESCRIPTION OF PLATES.

The figures are reproductions of photo-micrographs made by Mr. L. S. Brown, with the kind permission of Dr. J. H. Wright, of the Clinico Pathological Laboratory of the Massachusetts General Hospital.

All sections are from material fixed in Zenker's fluid, and stained with eosin polychrome methylene blue.

PLATE X.

Fig. 1. Clump of young, large mononuclear cells obstructing a capillary. They have displaced one or two liver cells, whose remains show as a light band near the mitotic figure. Liver of mouse No. 18, x 1000.

Fig. 2. Clump of large mononuclear cells lying in a capillary space. They have lost the power of staining with basic dyes, and show other degenerative changes. Necrotic liver cells at the periphery. A few polymorphonuclear cells among the phagocytes. Liver of mouse 17, x 1000.

Fig. 3. Area formerly occupied by phagocytic cell clumps. Now filled by a network of fibrin containing cell remains. Liver of mouse 5, x 250.

PLATE XI.

Fig. 4. Area of coagulation necrosis due to thrombosis of the portal vein by a hyalin thrombus. Liver of mouse 2, x 50.

Fig. 5. Parietal thrombus of the hepatic vein. Thrombus composed of agglutinated and partially fused red corpuscles. Many phagocytic cells in the vein. Liver of mouse 2, x 250.

Fig. 6. Area of coagulation necrosis in the liver of a rabbit due to thrombosis. A small hyalin thrombus in a hepatic vein, x 20.

ON THE MULTIPLICITY OF THE SERUM HEMAGGLUTININS OF COLD-BLOODED ANIMALS.[1]

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In the course of my studies of the action of the blood sera of cold-blooded animals upon the erythrocytes of those animals[2] I took account of the coincident occurrence of agglutination. At the conclusion of the experiments upon those subjects Professor Flexner suggested that I study the heat lability of the agglutinins for erythrocytes of cold-blooded animals, and also their behavior towards absorption tests. This I did in a few instances, the results of which I have thought of sufficient interest to warrant publication in a brief form.

The experiments upon heat lability were conducted with the serum of *Limulus polyphemus* — the horseshoe crab. The clear serum obtained from the body cavity is highly agglutinative for the corpuscles of many cold-blooded animals. The species used and the results obtained are given in Table I.

TABLE I.

Agglutinating Power of Normal Limulus Serum.

Species of blood.	Mustelus canis.	Emys meleagris.	Brevoortia tyrannus.	Paralichthys dentatus.	Tautoga onitis.	Cynoscion regalis.
Degree of agglutination.	Strong: 5 min.	Strong: 5 min.	Strong: 8 min.	Mod. strong: 10 min.	Strong: 5 min.	Moderate: 15 min.

The serum of *Limulus* was now heated for thirty minutes to temperatures varying from 45° to 65° C., after which the agglutination was again noted. The results are given in Table II.

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[1] Received for publication Feb. 9, 1903. This study was conducted under a grant from the Carnegie Institution of Washington, D.C.

[2] Univ. of Penn. Med. Bulletin, 1902, xv, 295, 301.

TABLE II.

Serum of Limulus Heated for Thirty Minutes.

Species of blood.	40° C.	45° C.	50° C.	55° C.	60° C.	65° C.
Mustelus canis.	Mod. strong	Mod. strong	Moderate	Weak	None	
Emys meleagris........	Strong	Moderate	Weak	None		
Brevoortia tyrannus......	Moderate	Weak	Weak	None		
Paralichthys dentatus	Moderate	Trace	None			
Tautoga onitis	Strong	Mod. strong	Weak	Trace	None	
Cynoscion regalis........	Moderate	Moderate	Weak	Very weak	Trace	None

The absorption experiments were made with *Limulus* serum and were conducted by adding to the serum an excess of the washed corpuscles of the several species of animals. These were allowed to remain in contact for one hour, after which the separation of corpuscles and serum was made by centrifugalization. The serum was now treated with corpuscles of another species in the same manner. The results are given in Table III., in which twenty cubic centimeters of the serum of *Limulus* has been employed.

TABLE III.

First Fraction : Serum of Limulus Treated with Corpuscles of Mustelas Canis.

Supernatant plus blood of......	Mustelus.	Emys.	Brevoortia.	Paralichthys.	Tautoga.
Agglutination.....	None.	Strong: 5 min.	Strong: 10 min.	Strong: 15 min.	Strong: 5 min.

Second Fraction: Serum of Limulus Treated with Mustelus and Brevoortia Corpuscles.

Supernatant plus blood of......	Brevoortia.	Emys.	Paralichthys.	Tautoga.
Agglutination.....	None.	Strong: 8 min.	Strong: 15 min.	Strong: 8 min.

Third Fraction: Serum of Limulus Treated with Mustelus, Brevoortia, and Emys Corpuscles.

Supernatant plus blood of.......	Emys.	Paralichthys.	Tautoga.
Agglutination	None.	Strong: 20 min.	Strong: 10 min.

Fourth Fraction: Serum of Limulus Treated with Mustelus, Brevoortia, Emys, and Paralichthys Corpuscles.

Supernatant plus blood of...............	Paralichthys.	Tautoga.
Agglutination	None.	Strong: 15 min.

Fifth Fraction: Serum of Limulus Treated with Mustelus, Brevoortia, Emys, Paralichthys, and Tautoga corpuscles.

Supernatant plus blood of	Tautoga.
Agglutination......................	None.

The serum of *Limulus* is well adapted for the experiments upon agglutination as it is almost without hemolytic power. Similar experiments conducted with the serum of *Mustelus canis* are of interest, perhaps, but are not free from the serious drawback that comes from the necessity of first heating the serum to destroy its hemolytic complement. However, after heating to 50° C. the serum shows agglutinating power over the corpuscles of *Amphiuma means*, *Chelydra serpentina*, and *Pleuronectes Americanus*. The abstraction of the agglutinin for corpuscles of one species leaves those for the corpuscles of others in practically undiminished quantities.

On the basis of the experiments given the following conclusions seem warranted:

1. The serum of *Limulus polyphemus* contains several and perhaps many agglutinins which are, in part at least, specific for certain erythrocytes.

2. The agglutinins show varying degrees of heat lability, although temperatures of 40° C., when continued for thirty minutes, diminish the activity of all the agglutinins. Temperatures approaching 65° C. seem to destroy wholly the agglutinating power of the serum for erythrocytes.

3. The complete absorption of agglutinins for the corpuscles of one or several species of animals from *Limulus* serum leaves the remainder of the agglutinins in almost undiminished quantities. A slight difference, in the case of certain erythrocytes, has been noted in the rapidity with which the reaction is completed in the serum from which a part of the agglutinins has been removed.

4. The serum of *Mustelus canis*, in so far as its agglutinins are concerned, agrees in its action probably with that of *Limulus*.

5. *Limulus* and *Mustelus* sera contain a multiplicity of agglutinins for erythrocytes of cold-blooded animals.

ON THE HEAT LABILITY OF THE COMPLEMENTS OF COLD-BLOODED ANIMALS.*

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The study of serum complements of warm-blooded animals has led to conflicting views upon their nature. According to some — Buchner,[1] Wilde,[2] Bordet,[3] Gruber[4] — they are unitary bodies which, as alexines, act upon susceptible cells,— bacteria, body-cells,— and bring about their dissolution. According to this view a given serum contains a single complement or alexine, which is brought into combination with the foreign cells through the operation of certain intermediate substances,— substance sensibilisatrice, amboceptor,— which differs for different kinds of cells. According to another view, that held by Ehrlich, his co-workers and supporters, the complements are pluralistic bodies which differ in their combining capacities, whereby their action is restricted within definite limits, as determined by this combining affinity with particular amboceptors. A given serum may contain several or even many complements, and these in turn may exhibit, besides the distinctions of combining power with amboceptors, other differences, as in respect to the resistance to the injurious action of heat, of acids, alkalis, and digestive ferments, and also as regards their ability to pass through unglazed porcelain filters.

In the course of experiments upon the hemolysins of cold-blooded animals† carried out last summer a few experiments were conducted to test the variations in heat lability of the serum complements of a small number of the species used for the experiments. The results are of interest in themselves and bear upon the broader question of the multiplicity of serum complements, wherefore they are presented at this time.

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* This study was conducted under a grant from the Carnegie Institution of Washington, D.C. Received for publication, Feb. 9, 1903.

† Noguchi. Univ. Penn. Med. Bulletin. 1902, xv, 205, 301; Centralb. für Bakteriologie, etc., 1903, xxxiii, 1 Abt., 353, 362.

For the purpose of the experiments to be given, the sera of four species of animals were employed. These were: *Mustelus canis* (dog fish), *Cynoscion regalis* (squeteague), *Amphiuma means* (Congo eel), and *Rana catesbiana* (bull-frog).

The fresh serum of *Mustelus canis* showed the following hemolytic action when used in the proportion of 1 cc. of fresh serum and 5 per cent of defibrinated blood:

Blood of *Rana catesbiana* . . .	Hemolysis:	15 minutes
" " *Amphiuma means* . . .	"	40 "
" " *Cynoscion regalis* . . .	"	5 "
" " *Chrysemys picta* (painted turtle)	"	45 "
" " *Emys meleagris* (box-tortoise)	"	10 "

The fresh serum of *Cynoscion regalis* used in the same manner gave the following result:

Blood of *Rana catesbiana* . . .	Hemolysis:	10 minutes
" " *Amphiuma means* . . .	"	40 "
" " *Chrysemys picta* . . .	"	30 "
" " *Emys meleagris* . . .	"	20 "

The fresh serum of *Amphiuma means* gave the following result:

Blood of *Rana catesbiana* . . .	Hemolysis:	15 minutes
" " *Chrysemys picta* . . .	"	25 "
" " *Emys meleagris* . . .	"	45 "

The fresh serum of *Rana catesbiana* gave the following result:

Blood of *Cynoscion regalis* . . .	Hemolysis:	5 minutes
" " *Mustelus canis*	"	1 "
" " *Tautoga onitis* (Tautog),	"	2 "
" " *Brevoortia tyrannus*(Menhaden)	"	12 "

For the purpose of our experiments the blood sera were heated to temperature of 40 degrees, 45 degrees, and 50 degrees for thirty minutes, after which the hemolytic action was again tested.

Experiment I.

Serum of *Mustelus canis* heated for thirty minutes.

5% blood of	40° C.	45° C.	50° C.	Unheate serum.
	Hemolysis.			
Rana catesbiana	35 min.	None		15 min.
Amphiuma means	42 min.	None		40 min.
Cynoscion regalis	25 min.	None		5 min.
Chrysemys picta	52 min.	Partial, 1 hour	None	45 min.
Emys meleagris	Partial, 1 hour	None		10 min.

Experiment II.

Serum of *Synoscion regalis* heated for thirty minutes.

5% blood of	40° C.	45° C.	50° C.	Unheated serum.
	Hemolysis.			
Rana catesbiana	35 min.	None		10 min.
Amphiuma means	Imperfect, 60 min.	None		40 min.
Chrysemys picta	.40 min.	Trace	None	30 min.
Emys meleagris	None			20 min.

Experiment III.

Serum of *Amphiuma means* heated for thirty minutes.

5% blood of	40° C.	45° C.	50° C.	Unheated serum.
	Hemolysis.			
Rana catesbiana	None			15 min.
Chrysemys picta	Partial, 1 hr.	Trace, 1 hr.	None	25 min.
Emys meleagris	Partial, 1 hr.	None		45 min.

Experiment IV.

Serum of *Rana catesbiana* heated for thirty minutes.

5% blood of	40° C.	45° C.	50° C.	Unheated serum.
	Hemolysis.			
Cynoscion regalis	25 min.	None		5 min.
Mustelus canis	30 min.	Trace	None	1 min.
Tautoga onitis	Trace	None		2 min.
Brevoortia tyrannus	20 min.	30 min.	None	12 min.

CONCLUSIONS.

1. The serum-complements of cold-blooded animals are highly labile bodies and are destroyed at temperatures below 50° C.

2. The serum-complements of these animals would appear to be multiple in that their action upon different species

of red corpuscles is not only different, but the destruction of the complement for one kind of red corpuscle may be effected without loss of the entire complement-content of the serum.

3. The complements for given kinds of red corpuscles can be greatly reduced without being entirely destroyed by heating the serum. When such reduction is brought about a slightly higher temperature completely abolishes the action of the complements.

4. The complements occurring in different sera show, for a given species of red corpuscle, different degrees of heat lability.

(I wish to express my indebtedness to Professor Flexner for his kindness in suggesting this study to me.)

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THE PERIOD OF DEVELOPMENT, THE TIME OF GREATEST ACCUMULATION, AND THE PERSISTENCE OF DIPHTHERIA ANTITOXIN IN THE BLOOD OF A SERIES OF ONE HUNDRED HORSES.[1]

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The maximal capacity of horses to develop antitoxin varies very greatly and depends to a certain extent only upon the amount of toxin injected and the frequency of the injections. This variation is of interest both to the biologist and to the producer of antitoxic sera for medical purposes. The following statements and tables are based on the experience derived by us from the injection of about two hundred horses with diphtheria toxin.

There seems to be a maximal antitoxic limit for each horse, beyond which it cannot be pushed, no matter what the amount of the diphtheria toxin injected. After reaching this maximal limit, which by our methods is attained on the average four months after the toxin injections begin, the blood serum may continue for a while, if the injections are continued, to contain antitoxin of the maximum amount, but sooner or later the quantity of antitoxin surely diminishes. It is seldom that a horse whose power of developing antitoxin, after having been pushed to the maximum, has diminished, can be stimulated to develop again the maximum amount of antitoxin. Probably horses 7 and 83 are only apparent exceptions to this rule as the notes below the table will show, since the injections were not pushed in the first months as high as required to develop the greatest amount of antitoxin. But in these cases, also, the horses' ability to develop antitoxin diminished after a time, until two hundred units per cubic centimeters was the best that could be obtained. The effect of a long rest in the pasture on a good antitoxin horse

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whose power of developing antitoxin has run down has been tried by us. Such horses rarely produced again their former maximum amount.

The Health Department of the City of New York has from March, 1897, until May, 1902, made use of one hundred horses for the production of diphtheria antitoxin. A consideration of the earlier horses is omitted because the methods used to develop the antitoxin were then variable.

These horses were carefully selected, and were all young, healthy animals of medium grade, and did not represent any special breed or locality.

In the majority of the horses the toxic effects of the first three or four toxin doses injected were more than neutralized with diphtheria antitoxin. The size of the first injections varied considerably, the earlier horses receiving three hundred to fifteen hundred guinea-pig fatal doses of toxin with but little antitoxin, the later horses receiving five thousand to forty thousand guinea-pig fatal doses of toxin fully neutralized. With each succeeding injection the antitoxin amount was gradually diminished.

The toxin injections were increased each time by as much as was considered safe, usually twenty-five to fifty per cent, and repeated as soon as the temperature fell to the normal, about every five to seven days.

The following table is a summary of our data:

TABLE I.

Horses producing diphtheria antitoxic sera. 100 consecutive horses tested.	Below 300 units per cc.	300–400 units per cc.	400–500 units per cc.	500–600 units per cc.	600–700 units per cc.	700–800 units per cc.	800–900 units per cc.	1000–1100 units per cc.	1200–1300 units per cc.
Number of horses producing diphtheria antitoxic sera. (Maximum strength)	51	12	11	9	7	7	(*horse 182*) 1	(*horse 112*) 1	(*horse 137*) 1
Average of guinea-pig fatal doses given to produce maximum antitoxic strength.	666,316*	1,027,067	1,260,000	1,338,666	725,564	1,367,362†	412,000	1,082,500	354,200
†Period of injections.	3 months*	3.85 months	4.55 months	4.55 months	5 months	4.64 months	2½ months	10 months	2½ months
Average length of time horses continued to produce diphtheria antitoxic sera of 300 units per cc. or greater.		1 bleeding only except in the case of horse 174, which gave two bleedings of 300 units per cc.	2 months	3 months 27 days	6 months 24 days	5.3 months‡	14 months§	23 months and 18 days.	5 months 6 days‖
Per cent of different grades of sera.	51	12	11	9	7	7	1	1	1

Per cent of horses producing serum below 300 units per cc., 51.
" " " " " over 300 " " 49.
" " " " " " 400 " " 37.
" " " " " " 500 " " 26.
" " " " " " 600 " " 17.
" " " " " " 700 " " 10.
" " " " " " 800 " " 3.

Fourteen of the 51 horses recorded as giving sera below 300 units per cc. died before a test for strength had been made of their sera, but they are placed in the list because at the time of death it was too early to expect a serum containing 300 units per cc.

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*The small amount of toxin injected, and the short period during which the injections were carried out, were due to our having gotten rid of the horses when they failed to increase in antitoxin.

†Horse 184 required 5,214,000 guinea-pig fatal doses, and horse 193 required 1,131,500 guinea-pig fatal doses to develop the greatest immunity. The other five horses of the 700–800 unit grade averaged 648,140 guinea-pig fatal doses to develop the greatest immunity. Horses 184 and 193 bring up the average of the time required to develop the greatest immunity of their grade from 3.3 to 4.64 months.

‡One of these horses is still producing antitoxin above 300 units.

§Still producing antitoxin above 300 units.

‖The early death of 137 was caused by an accident, and was not due to the toxin injections. 137 was still producing high-grade antitoxin.

It will be seen by the table that forty-nine per cent of these horses were capable of developing antitoxic sera of three hundred units per cubic centimeter or more, and that twenty-six developed antitoxic sera of over five hundred units per cubic centimeter, the so-called high-grade sera.

Eleven horses which failed to develop three hundred unit sera received enormous total toxin injections, varying from 1,195,250 guinea-pig fatal doses to 3,005,500 guinea-pig fatal doses, which covered a period of three to six months; average, four and one-half months.

The horses incapable of developing sera containing three hundred units per cubic centimeter were replaced by others with the hope of getting stronger sera. This, together with the early deaths of fourteen horses, accounts for the short period they were kept and for the smallness of the average of total guinea-pig fatal doses they received.

It is interesting in Table 1 to note the great variation in the average number of guinea-pig fatal doses and the average period of injections required to produce the maximum strength in the different grades of horses. The figures are somewhat misleading where single horses are concerned, for the reason that they may have been bled and their injections for several weeks stopped before reaching the maximum amount of antitoxin.

A few notes on some of the horses that have given the largest amount of antitoxin may be of general interest.

Horse 112 was such a remarkable antitoxin producer, that a table showing the series of toxin injections, the number of bleedings, and the strength of the sera obtained, beginning with the first series of toxin injections and ending at the point of greatest immunity, is given as the clearest way of setting forth these facts.

TABLE II.

Chart of the Series of Toxin Injections and Bleedings and Strengths of the Sera Obtained from Horse 112.

Total number g. p. f. d. at time of bleeding....	180,000 g. p. f. d.	330,000 g. p. f. d.	450,000 g. p. f. d.	767,000 g. p. f. d.	1,057,000 g. p. f. d.	1,187,000 g. p. f. d.	1,847,000 g. p. f. d.
Dates of bleedings..........................	X. — 15 1896	XI. — 18 and XII. — 5	XII. — 30 and I. — 5, '97	II. — 9 and II. — 19	III. — 26	IV. — 19	V. — 25 and VI. — 1
Strengths of sera in units per cc.............	500 units	700 and 450 units	800 and 700 units	700 and 600 units	600 units	700 units	1,000 and 600 units

Had it not been necessary to bleed horse 112 so early and so constantly, the point of greatest immunity would undoubtedly have been reached much sooner and with many less guinea-pig fatal doses. For fifteen months after developing its maximal antitoxic strength horse 112 continued to yield sera above three hundred units per cubic centimeter. The strength of the serum then fell to about two hundred units per cubic centimeters, above which it did not rise again.

Horse 137 after the initial bleeding (1,200 units per cc.) never gave a stronger serum than six hundred units per cubic centimeters, but held at that strength until its death. Two horses not included in this series are worthy of mention.

Horse 83 after receiving 361,000 guinea-pig fatal doses gave a five hundred to six hundred unit serum (period of injection, 2¼ months).

After receiving 2,904,000 guinea-pig fatal doses additional, the serum did not increase in strength more than one hundred units per cubic centimeter.

After a rest of one and one-fourth months the horse was given 1,115,000 guinea-pig fatal doses. The strenth of the serum rose to eight hundred and fifty units per cubic centimeter. As in the case of horse 112 had it not been necessary to constantly bleed horse 83 the maximal antitoxic strength probably would have been reached much sooner. Number 83 furnished antitoxic sera usually above five hundred units per cubic centimeter through a period of twenty-two months.

One of the first horses treated, number 7, was bled at intervals for four years and gave a serum varying between three hundred and five hundred units. The injections in this horse were not pushed to the limit. This horse is not included in Table I.

We may conclude from the above data that from carefully selected young horses of medium grade,

1. About one-half will develop sera of three hundred units strength per cubic centimeter or greater, and that about one-fourth will develop sera of five hundred units strength per cubic centimeter or greater.

2. That the average development of maximum strength may be expected three to five months after the first toxin injection, according to the rapidity with which the toxin is pushed.

3. That horses capable of producing a high grade antitoxin serve a longer period of usefulness than horses capable of producing a medium grade antitoxin.

(In conclusion, I wish to thank Dr. William H. Park, Director of the Research Laboratory, for his kindly criticism, and Dr. George P. Biggs, Assistant Pathologist, for his help in supplying me with much of the data.)

THE PRESENCE OF THE SHIGA VARIETY OF DYSENTERY BACILLI IN AN EXTENSIVE EPIDEMIC OF DYSENTERY WITH NOTES UPON THE SERUM REACTIONS OBTAINED.[1]

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An extensive epidemic of dysentery occurred in the small town of Tuckahoe, situated just north of New York City, during the past summer. Cultures were made at the time from a fatal case (Carey), and later the agglutination characteristics of the culture studied and the serum reactions tested in a number of the convalescent cases (Park).

Although the information here gathered is limited in amount, yet it is published because the epidemic has ended and it is thought that the results obtained add something of value to the information already gathered on the subject of the relation of a rather ill-defined group of bacilli to epidemic dysentery.[2]

The following are some of the most essential points concerning the epidemic. Until July, 1902, there had been very little dysentery in Tuckahoe (a town of some 3,000 inhabitants), but during July, August, and September of that year there were about three hundred and fifty cases, with a mortality of about six per cent. The average age in the fatal cases was a little less than three years. The epidemic was most severe among a class of people living in filthy surroundings. There was in this region no system of sewerage, the old-fashioned out-of-door closets being in general use. Chemical examination showed the well water to be badly

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[1] Received for publication Feb. 1, 1903.

[2] A full account of the territory covered by the epidemic and of the clinical course of the disease in the cases can be found in the N. Y. University Bulletin of the Medical Sciences, Vol. 2, No. 4, October, 1902, by Park and Dunham.

contaminated. The epidemic in a less severe form spread to the districts adjacent to Tuckahoe where the water supply and sewerage were good.

The chief points of clinical interest in regard to the cases as summarized from details furnished by Drs. Tupper and Austin, two physicians of Tuckahoe, who treated over two hundred and fifty of the persons attacked, are as follows:

The onset in all of the cases was sudden. The disease was ushered in by cramps, diarrhea, and tenesmus. The stools were at first feculent, then sero-mucous, then finally bloody and mucous. Blood usually appeared on the second day, was present in considerable quantity for two or three days, and then diminished. It disappeared entirely in most cases in six to ten days.

Temperatures were low; if there was no complication the average case ran from 99° to 101° F. for one or two days, then fell to normal or sub-normal. Sub-normal temperatures were very frequent. Tenesmus was a marked feature of almost every case. A few cases developed rectal prolapse. The stools had the characteristic cadaveric odor, and were composed of mucus and blood, frequently mixed with fragments of mucous membrane. A stool frequently seen was composed of "coffee ground" sediment, serum, and mucus. The stools usually consisted of a small amount of mucus frequently streaked with blood. Pure bloody evacuations occurred in most of the cases. These discharges only lasted twenty-four to forty-eight hours as a rule, beginning on the second day. Mild cases in general lasted two weeks, severe three to four weeks, malignant a few days. Convalescence was slow almost without exception.

The autopsy records of the fatal case, from which the dysentery bacillus (Shiga type) was obtained are as follows:

Harriet T. · Age, one year and four months. Died August 26, at 7 A.M. Autopsy, 1 P.M. Service of Dr. Oliver Austin, Tuckahoe, N.Y.

Mucous Membranes. — Pale. Edema of feet and legs.

Peritoneum. — On opening abdominal cavity both layers of peritoneum are smooth. No adhesions. No distension with fluid.

Intestines. — Not discolored.

Small intestines. — Slightly distended. Mesenteric glands large and red, vary in size from 3 to 6 mm. in diameter.

Large intestines. — Outer surface vessels congested and prominent. On section covered with a yellowish mucus. Mucous membrane seems to be absent in places. Solitary follicles are elevated and enlarged, especially in the region of sigmoid flexure. In some instances the center of the follicles are depressed and appear to be necrotic.

Appendix. — No adhesions on the outer surface. On section lymphatic follicles are swollen with depressed centers similar to the condition described in the large intestines.

Small intestines. — Peyer's patches are distinctly swollen, but in no instance is there ulceration or necrosis.

Kidneys. — Fibrous capsule not thickened and peels easily. On section cortex not thickened. Pyramids distinct. Malpighian corpuscles not visible. Blood vessels congested. Kidney substance bleeds easily.

Liver. — Outer surface smooth. Consistency soft. Capsule not thickened. On section lobules are distinct.

Spleen. — Normal in size. Capsule not thickened. On section Malpighian corpuscles distinct. Pulp not increased. Bleeds easily on section.

ANATOMICAL DIAGNOSIS — Passive congestion of the lungs, spleen, kidneys, and liver. Follicular enteritis. Hyperplasia of the mesenteric lymph glands.

MICROSCOPICAL EXAMINATION.

Liver. — Cells of parenchyma normal. Marked congestion of the hypatic vein and capillaries at center of lobule.

Spleen. — Slight congestion.

Heart muscle. — Normal.

Kidney. — Cloudy swelling.

Lung. — Marked congestion of lung capillaries.

Thymus. — Marked congestion with considerable accumulations of eosinophilic cells, mostly polynuclear.

Large intestine. — Mucosa glands are for the most part normal, but over the solitary follicles they have broken down somewhat and contain polynuclear leucocytes. The interglandular stroma in these places has undergone necrosis. The necrotic areas extend down into the submucosa in the region of the solitary follicles. The blood capillaries of the solitary follicles are much dilated and congested. The submucosa is thickened and slightly edematous. The connective tissue cells appear to have undergone a slight hyaline degeneration. The musculature is not affected, neither is the peritoneal coat.

Small intestines. — Normal.

Large intestine. — In section stained by Flexner's method bacteria were demonstrated in the necrosed areas which corresponded in shape and size to those of the colon group.

AUTOPSY BACTERIOLOGY.

Cultures were made from the lungs, heart, liver, spleen, kidneys, and large intestine.

Heart, spleen, and kidneys. — Sterile after 72 hours' incubation.

Liver. — B. Coli Communis.

Large intestine. — A series of twelve agar plates were made and placed in the thermostat at 37° for 24 hours. At the end of that time all the colonies were marked and cultures from those chosen for inoculation subsequently proved to be B. Coli Communis.

After a second incubation of 24 hours new colonies developed, some of which were inoculated into fermentation tubes containing glucose bouillon.

After 24 hours several tubes contained gas, and the bacilli in them proved to be colon bacilli. The culture in one tube, however, developed no gas after 48 hours, and subcultures were made upon different culture media.

The cultural characteristics of this organism are as follows:

Agar. — Slight growth along line of needle, white, opaque, elevated, and moist.

Milk. — Slight acid formation in first two days. Alkalinity developed after ten days.

Neutral red. — No change.

Bouillon. — Clouded.

Dunham's peptone solution. — Very slight indol formation at end of five days.

Gelatin. — Not liquefied.

Glucose Bouillon. — No gas.

Mannit nutrient agar. — No production of acid in deep stab or surface growth.

Lactose bouillon. — No gas.

Saccharose bouillon. — No gas.

Morphology. — Short bacillus, rounded ends. Sometimes in pairs.

By Gram. — Decolorized.

Motility. — Very slight, if present at all.

Flagella. — Not demonstrated.

Agglutination Reactions. — The reaction of the Tuckahoe bacillus with the serum from a horse which had received seven subcutaneous injections of Flexner's Baltimore culture compared with Shiga's Japan culture and Flexner's Baltimore culture is as follows:

Table showing agglutination of Tuckahoe culture and of example of Shiga and Flexner Baltimore types in horse immunized to Baltimore culture.

Dilutions	1-50	1-100	1-250	1-500	1-1000	1-2000	1-4000
Shiga bacillus	++	+	+	—	—	—	—
Flexner's Baltimore bacillus	++	++	++	++	+	—	—
Tuckahoe bacillus..	++	+	+	—	—	—	—

++ = complete clumping reaction, + = incomplete. Time, 2 hours.

In one sense all these cultures gave a positive serum reaction, and the difference between the reactions of the Flexner and the Shiga and Tuckahoe cultures in this serum appears from these results to be a quantitative rather than a qualitative one. That this is not true is shown by the fact that normal horse's serum has in our experience frequently clumped all these cultures in 1–250 dilutions. A young goat's serum which before injection with cultures did not agglutinate any of the varieties of dysentery bacilli in our possession gave the following results after seven injections:

Table showing agglutination reaction in a serum having only specific agglutinins.

Dilutions	1-50	1-100	1-250	1-500	1-1000	1-2000	1-4000
Shiga bacillus, Flexner's New Haven Our Tuckahoe...	—	—	—	—	—	—	—
Flexner's Manilla, Baltimore, etc., Our Seal Harbor, and N.Y. cultures,	++	++	++	++	++	+	—

++ = complete agglutination, — = no agglutination of bacilli. Time, 2 hours.

It is a striking fact that all the varieties of dysentery-like bacilli obtained from dysentery cases which produce indol in large amount and develop acid from mannit agglutinate alike, while those which produce no indol or only a trace and do not develop acid from mannit also group themselves together in so far at least as not reacting to some sera which powerfully agglutinate the others.

THE SERUM TESTS OF THE BLOOD OF CONVALESCENT DYSENTERY CASES FROM TUCKAHOE. — Information concerning the extensive epidemic of dysentery only reached one of us on his return to New York, in October. No acute cases remained and no dysentery-like bacilli were found in feces of several convalescents examined.

The finding of the bacillus of the Shiga type in a fatal case being then unknown, the serum tests were carried out with the Flexner Manilla type, to which type the bacilli obtained by us in New York City belonged.

The serum reaction was positive within one and one-half hours in a 1–50 dilution in thirteen out of twenty-two cases. The agglutinating strength shown was much above the average found by us in the serum of those who had not recently suffered from dysentery. Tests made two months later from some of the same cases showed a marked reduction in the agglutination strength of the sera of these cases. Although the results of these tests are insufficient to prove that the mannit fermenting type of bacilli was present and active in these cases, still they strongly suggest this to be so.

If this conclusion is correct, then bacilli of both the mannit fermenting and Shiga type were present in the cases. The Shiga variety was shown to be present by finding it in a fatal case, and the other's presence was suggested by the positive serum reactions obtained with the Baltimore culture in serum from the dysentery cases after their recovery. This proof was strengthened by the rapid disappearance of the substance causing the agglutination as shown by tests made two months later. When tested at this time the reaction between both varieties of bacilli and the sera was nearly equal in amount.

The following tables give the serum reaction of the blood of a number of dysentery cases two months and five months after recovery.

SERUM AGGLUTINATION TESTS WITH BLOOD OF TUCKAHOE DYSENTERY CASES, TAKEN TWO AND FOUR MONTHS AFTER DISAPPEARANCE OF SYMPTOMS.

Case.	Blood taken 2 mos. after convalescence. Reaction with Baltimore culture.			Blood taken 4 mos. after convalescence. Reaction observed in diluted serum at 1½ hrs. in hanging drop.										
				Flexner Baltimore Culture.			Tuckahoe Culture.			Shiga's Japan Culture.			Colon Bacillus.	
	1-10.	1-20.	1-50.	1-10.	1-20.	1-50.	1-10.	1-20.	1-50.	1-10.	1-20.	1-50.	1-10.	1-20.
4	+ |	+	+	+	—	—	+	|	—	+	—	—	+	—
5	+ +	+ +	+	+	+	—	+	+ |	—	+	+	—	|	—
6	+ |	+	+	+	+	—	+	|	—	+	—	—	|	—
14	+ |	+	+	+	—	—	—	—	—	—	—	—	|	—
17	+ |	+	+	+	+	—	+	|	—	+	—	—	+	|
20	+ |	+	+	|	—	—	+	—	—	+	—	—	—	—

No. Case.	Age.	Date of Illness.	Date of Test.	Reaction.					Severity of Disease.			Symptoms.		No. in Family.	No. Having Dysentery.
				1½ Hours.			24 Hours.								
				1:10.	1:20.	1:50.	1:20.	1:50.	Severe.	Mod.	Mild.	Char. Dyst.	Simp. Diar.		
1........	16 years.	Aug.	Nov.	+	—	—	+	—	+			+		2	1
2........	16 "	"	"	++	+	+	++	++		+		+		9	1
3........	9 "	"	"	—	—	—	—	—		+		+		9	4
4........	17 "	Sept.	"	+|	+	+	+	+		+		+		8	1
5........	—	Aug.	"	++	++	+	+	+			+	+		-	-
6........	9 "	"	"	+|	+	+	++	++			+	+		5	2
7........	32 "	"	"	—	—	—	—	—			+	+		7	7
8........	3 "	"	"	—	—	—	—	—	+			+		7	7
9........	38 "	"	"	+|	+	+	++	++		+		+		7	4
11........	34 "	Sept.	"	—	—	—	—	—	+			+		3	2
12........	36 "	Aug.	"	++	++	++	+	+			+	+		4	2
13........	8 "	"	"	++	++	++	++	++	+			+		4	2
14........	41 "	"	"	+|	+	+	+	+			+	+		7	6
15........	13 "	"	"	—	—	—	—	—			+	+		7	6
16........	11 "	Sept.	"	+|	+	+	+	+	+			+		7	6
17........	4 "	"	"	+|	+|	+	+	+	+			+		7	6
18........	9 "	"	"	++	++	++	++	++	+			+		5	1
19........	2 "	"	"	+|	+	—	+	—		+		+		4	1
20........	5 "	"	"	+|	+	—	+	—	+			+		7	3
21........	—	Aug.	"	—	—	—	+	—	+			+		-	-
22	11 "	"	"	+|	++	++	++	++	+			+		-	-

+ indicates good reaction, but with small clumps and with a few separate bacilli.
+| indicates almost complete clumping of all bacilli.
++ indicates complete clumping of all bacilli.
— indicates little or no clumping.

SUMMARY.

From a fatal case of dysentery, occurring in a severe and extensive epidemic in a small town situated just north of New York City, a bacillus was obtained which, in all but one of many cultural tests, seemed identical with Shiga's Japan and Flexner's New Haven culture. The only point of difference being that the Tuckahoe culture produced a trace of indol in peptone solution. The specific agglutinins developed in animals through injection of the group of "dysentery" bacilli, which produces considerable indol and develops acid from mannit, did not agglutinate the Tuckahoe, Shiga, or New Haven cultures. All bacilli so far obtained in America (Flexner's Baltimore and Philadelphia cultures, and ours from New York City and Seal Harbor), outside of the New Haven and Tuckahoe cultures, seem to belong to this class.

The serum reactions of the blood taken from a number of convalescent cases with the Baltimore culture were on the average much more marked than those obtained by us from the blood of acute cases of other intestinal diseases, and indicate that this variety of bacilli, as well as the Tuckahoe variety, was present and active in many of the Tuckahoe dysentery cases. Three months later the serum reaction had largely disappeared in the cases tested, and was about alike for both varieties of bacilli.

CONCLUSIONS.

At least two distinct varieties of bacilli have been found in the intestines of those suffering from acute dysentery and colitis, which are probably exciting factors in the disease. These two varieties, though resembling each other greatly in most cultural characteristics, yet differ in some (indol formation and mannit fermentation), and also in their agglutination reaction.

Though Martini and Lentz are right, therefore, in their statement that the Shiga type differs from Flexner's Manilla-Baltimore type in cultural and agglutination reaction, yet they appear to us to be wholly wrong in concluding, upon our present information, that one type of organism is a factor in dysentery and the other not. Indeed, bacilli such as those obtained by Deycke from dysentery in Constantinople, which approached the colon type in cultural characteristics, cannot as yet be overlooked.

It seems to us that it is extremely probable that Shiga, if he carries his cultural and agglutination tests as far as is now known to be necessary in order to differentiate the different varieties, will find that in Japan, as well as in America and elsewhere, not one but several varieties of this group of bacilli are present.

The use of more accurate agglutination tests in connection with additional culture media has brought to light the fact that in different epidemics the bacilli believed to have excited the disease vary so greatly from the original culture of Shiga that at present we can hardly decide whether a single group is broad enough to comprise all the varieties. It is perhaps more than a coincidence that in most of the epidemics of characteristic dysentery the bacilli found have not fermented mannit. Even if the serum treatment proves of great value we can hardly hope that the bactericidal properties obtained through the injection of animals with one type of culture will be useful in persons suffering from the other type.

(In closing, we wish to express our sincere thanks to Dr. Simon Flexner for his kindness in sending us his cultures, and to Dr. Katherine R. Collins for assisting in making the numerous serum tests required.)

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THE TOXICITY OF TETRA-PHOSPHORUS TRI-SULFID.*— A PRELIMINARY REPORT.

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(*Cornell University Medical College, New York.*)

Although phosphorus was introduced into manufactures early in the nineteenth century, and not long afterward began to be used against vermin in the well-known form of paste, it was not until about 1845 that cases of fatal poisoning by phosphorus in the human subject began to be collected. Since that time it has become a frequent means of suicide, especially among women and on the continent of Europe. The usual methods of taking phosphorus to produce death are the ingestion of match heads or of an infusion made from them, and swallowing a certain amount of phosphorus paste in some fluid vehicle.

According to Smita,[52] the average phosphorus content of a match head varies from 0.00178 gram to 0.000167 gram, but most commonly the variation is less, running from one-half to one milligram. If, therefore, a person swallows the heads of one hundred and eighty matches, or of two hundred and fifty matches, as in two cases of Krug's,[26] the actual dose of phosphorus may be as high as 0.09 to 0.125 gram.

The lethal dose of phosphorus is given by Kolisko[24] as ten to twenty centigrams, but Sonnenschein (cited in 24) reported a case of a child aged five weeks who sucked the head of a single match with fatal result. Vetter[59] gives the percentage of phosphorus in the usual samples of paste as from eight to fifty per cent, but as it is almost impossible to determine the amount of the paste taken, the actual dose cannot be accurately known.

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* Received for publication March 2, 1903.

With a view to ascertain the comparative toxicity of phosphorus tri-sulfid (P_4S_3), which has been employed in the arts within the last decade, the following investigation was begun. The literature of phosphorus poisoning was examined and abstracted and several experiments upon animals were made with P_4S_3. But little experimental work has been done upon this sulfur compound of phosphorus, the most important papers being those of Courtois-Suffit and of Sevène and Blanc. The latter observers found no marked effects in guinea-pigs which received 0.03 gram of phosphorus tri-sulfid in repeated doses, though 0.003 gram of phosphorus was uniformly fatal. Their results will be noted in detail below.

Tetra-phosphorus tri-sulfid was given to two dogs in moderate morning and evening doses for a period of eight days, and, after a pause of one week, for a second period of eight days in the same manner. To a third dog single large doses were given daily for four days. A fourth dog received a single extremely large dose. In the sixth experiment a rabbit was exposed under a bell jar to a moist atmosphere which had been passed through several layers of the compound.

Dogs I. and II. received a weighed amount of food; the metabolism of fat, nitrogen, sulfur, and phosphorus was charted; the dogs were killed and immediately sectioned, and the organs were hardened in alcohol, formalin, or Orth's fluid, and examined after imbedding in celloidin or paraffin.

HISTORICAL.

In order to bring out the contrast between the effects of phosphorus and those of P_4S_3, it will be well to give a summary of the main facts in regard to poisoning by the former, the chief clinical symptoms and the various lesions found, and then discuss the observations made during this investigation.

As is well known, the poisonous form of phosphorus is the yellow, which is soluble in various media; the red form is non-soluble and non-toxic.

In the majority of cases of suicide, or murder, or accidental death by phosphorus, the onset and duration of the symptoms may be termed acute or sub-acute. The prominent symptoms are given by Lewin[30] as:

Severe gastric or abdominal pain in .	92.0 per cent.
Vomiting	81.2 " "
Ecchymoses and hemorrhages . .	45.0 " "
Icterus	34.1 " "
Diarrhea	30.0 " "

Other symptoms which are often encountered are dyspnea, convulsions, coma, paralysis, suppression of urine, fever, severe thirst, clay-colored stools, abortion, etc. Hamilton and Godkin[18] state that ten to fifteen per cent of all cases die within twenty-four hours, and various observers report death within a few hours. Many cases, however, last for several days, and there may be an interval of comparative comfort after the first sudden symptoms before the case again becomes serious.

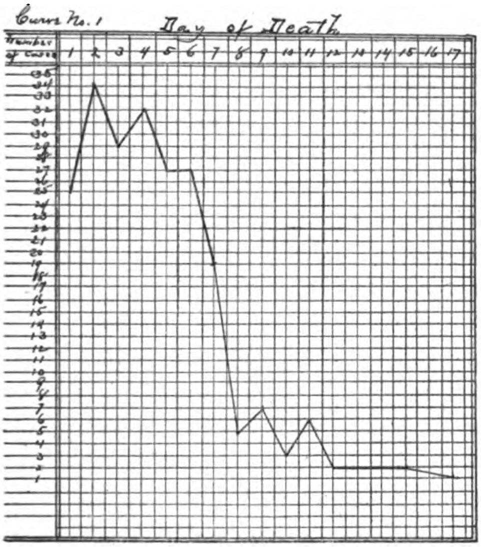
Taking the acute and the sub-acute cases together, death may occur in seven hours (Maschka), or as late as the seventeenth day (Hessler). The following partial table gives the variation in the time of death after taking the poison, with some other facts:

OBSERVER.	Sex.	Age.	Form and Amount.	Day of Death.
1. Maschka	Child.	3 wks.	3 match heads.	Seven hours.
2. Tüngel	F.	25 yrs.	Not given.	A few "
3. Kunstmann	F.	56 "	" "	24 "
4. "	F.	32 "	" "	30 "
5. Coupland	F.	14 mos.	Sucked matches.	44 "
6. Heberda	F.	21 yrs.	Not given.	3 days.
7. Maschka	F.	16 "	Match heads.	3 "
8. Burge	F.	15 "	100 match heads.	4 "
9. Pastau	F.	34 "	8 pkg. match heads.	5 "
10. Stumpf	F.	17 "	Not given.	5 "
11. Mialhe............	F.	12 "	" "	5 "
12. Grose.............	F.	5 "	Paste.	5 "
13. Vollbracht	F.	?	2 pkg. matches.	5 "
14. Krug	F.	15 "	180 match heads.	6 "
15. "	F.	15 "	250 " "	6 "
16. Leonpacher	F.	78 "	Not more than 0.06	7 "
17. "	F.	17 "	Not given.	8 "
18. Oppolzer..........	F.	24 "	6 pkg. matches.	8 "
19. Buchner	M.	Adult.	Rat poison.	9 "

Of two hundred and nineteen cases collected from the literature, one hundred and ninety-three, or 88.12 per cent, died within the first seven days, as will appear from the annexed curve. Of two hundred and eight fatal cases cited in Witthaus and Becker,[64] the shortest duration was seven hours, the longest was fifty-three days, and the average five and one-quarter days. The percentage of fatal cases is there estimated at about eighty per cent.

The effects of phosphorus in the organism may be considered with reference to the human subject or to animals, both subjectively and objectively during life, and in the lesions found post-mortem.

In from ten to fifteen per cent of the cases the onset of symptoms is rapid, but in the majority they appear only after several hours or days. There is a peculiar odor in the breath, there are nausea or vomiting, thirst, slight fever, diarrhea, and the vomitus and the feces may smell of phosphorus and glow in the dark. The feces may be clay colored or acholic, but as the color of normal feces depends upon the presence in the intestine of the products of both the liver and the pancreas, bile may be present even in seemingly acholic feces, if there be a lack of pancreatic juice in the intestine. The liver becomes large and tender. Epigastric and abdominal pain may be severe and continuous or intermittent. Icterus is common by the third day if the case live till then. Fever is seldom high, and toward the end the temperature may be subnormal. The pulse is usually feeble, and this may progress to complete cardiac



Curve showing day of death in 219 cases of phosphorus poisoning; 193, or 88.12 per cent, died within the first seven days.

failure, as might be expected from the fatty changes in the heart. Delirium and convulsions are not uncommon, and coma, if present, may resemble the uremic form.[57]

The urine is frequently either diminished or suppressed. Bettelheim[3] found a great increase in the alkaline phosphates in the urine. Free fat, albumin, blood cells, bile pigment, and both leucocytes and epithelia in fatty degeneration have been found in the urine. Sarcolactic acid,[18, 20] leucin, and tyrosin occur in the urine, and Bauer found paralactic acid. The urea is at first diminished, then increased, and afterward much decreased, with a corresponding increase in the liver, the muscles, and the brain.[57] Selmi found volatile phosphorous compounds in both the urine and the brain, and all were toxic.[48] Hemoglobinuria is described by Silbermann.[51]

Post-mortem the lesions are evident in a certain relation to the amount and form of the poison ingested and the duration of life afterward. The skin and conjunctivæ may be jaundiced and petechial hemorrhages in the integument are common. The muscle of the heart may be pale, or yellow and friable,[31] and the cardiac lesion may be seen as early as the third day.[19] The intima of the vessels may be in fatty degeneration, and rhexis of small vessels is common and may be general. Klebs found the vessels of the subcutis in fatty degeneration, and Orth and Heschl have described the same lesion in the brain. Hemorrhages, usually of small extent, may occur in any organ and in the muscles, including the tongue, heart, and uterus, and also under serous and mucous membranes. Extreme congestion of the liver, kidneys, ovaries, spleen, uterus, and other organs has been reported. Peripheral gangrene has been noted by Ehrlich, Heberda, Vollbracht, and others. The blood after death may be incompletely coagulated, which is explained by Corni and Ansiaux as due to lack of fibrinogen, fibrin ferment, and prothrombin. Heberda contradicts this view, though it is supported by experimental studies of Fränkel and Rohmann.

The lungs may not be altered apparently, but small hemorrhages under the pleura and throughout the organ are not uncommon. Wegner found fatty degeneration in the tissue of the lung in chronic cases. Puppe, Carrara, and others describe fatty embolism of the pulmonary capillaries. (Cited in 61.)

The spleen is variously described as firm and congested, enlarged and soft, or practically normal. The Malpighian bodies may be prominent on the fresh section. Hessler claims that seventy-five per cent of all cases have enlargement of the spleen.

The liver invariably shows lesions after toxic doses of phosphorus. Since Rokitansky in 1859 and Hauff in 1861 first called attention to the hepatic effects of phosphorus, all observers have agreed in describing fatty lesions of the organ in such cases. Some of the more enthusiastic have described in addition early and rapid proliferation of periportal connective tissue, a lesion developed experimentally over long periods by Wegner, but which in the rapidly fatal cases in the human subject may be as truly a coincidence as the arterial hypoplasia described by Manner in cases associated with gangrene of the extremities. In gross the liver may be large, with swollen and blunt edges. In consistence it may be firm or impressible, mottled red and yellow, congested, anemic (Burgl), or with only the average amount of blood (Heberda). Frequently it is stained with bile. In older cases there may be diminution in the size of the organ, indistinctness of the acini, and red areas where destruction of the cells has left the turgid vessels surrounded by detritus. This form resembles the liver of acute yellow atrophy. An excess of fat may be present early, distributed all through the acini or confined to the peripheries, where degeneration is apt to begin. Microscopically the amount of fat varies, but in the highest grade there is complete destruction of the cells. The bile capillaries may be found empty and pervious (v. Pastau), or plugged with mucus (v. Ebstein and Bollinger), and the mucus plug may have become hyaline. Kohts, and Munk and Leyden agree in ascribing the jaundice to extension of inflammation from the duodenum and plugging of the biliary capillaries. Meyer[85] found, beside occlusion of the finer biliary passages, plugging of the common bile duct also, and diffuse periportal proliferation of connective tissue.

The kidneys may be large. Cayley[8] in one case gives their weight as eleven and three-quarters ounces together. They may be congested or pale or yellow. The cortices are usually swollen and opaque, or distinctly yellow. Burgl[7] gives them as swollen but not opaque, with prominent glomeruli, in one of his cases. Jaundice and ecchymoses are often found in this organ. Microscopically, in early cases the epithelia of the convoluted tubules are fatty; later the cells of Henle's loops, of the straight tubes, and even of the capsules of the glomeruli may be fatty (Meyer, Virchow's Arch, xxxiii). The tubules may be more or less completely filled with desquamated fatty epithelia, leucocytes, which also may contain fat, bile pigment, and hyaline casts.

The mucous membrane of the stomach, apart from small hemorrhages, may be uniformly pale and swollen, cloudy and opaque, and white, gray, or yellow. Superficial erosions are described by some observers, but it is possible that post-mortem softening of the superficial layers of the mucosa may have been confounded with ante-mortem conditions. Microscopically the cells of the glandules are so swollen that their lumina are obliterated. These cells are opaque and granular, of a light yellow gray color by transmitted, and white by reflected, light. Acetic acid does not clear them. This lesion constitutes the gastradenitis phosphorica of Virchow[60] and is found in about one-half of the cases. It may be confined to either the pylorus or the cardiac end of the stomach, or be general.

The intestine may share in the inflammation or escape entirely. Superficial and punctate hemorrhages have been seen, Peyer's patches and the solitary follicles may be swollen, and the glands of Brunner and of Lieberkühn may resemble those of the stomach. The mesenteric nodes may be hyperemic and swollen (Coupland[11]).

The pancreas is usually in a condition of fatty degeneration.

One of the most recent studies of phosphorus, in relation to its effect upon metabolism in man, was published by Pecci.[41] He finds that in severe cases of acute phosphorus poisoning the nitrogen excreted as urea is decreased; that the nitrogen excreted as ammonia is increased, in direct proportion to the amount of proteid destruction in the body; and that the nitrogen lost as uric acid is increased both absolutely and relatively. The last of these observations is explained as due in part to destruction of tissue rich in nuclein. The phosphates in both urine and feces are increased, the chlorides vary with the amount ingested in the diet. The sulfates vary with the degree of proteid destruction, keeping pace with the nitrogen. The conclusion is reached that phosphorus causes a great increase in catabolism, and that there is an active formation of ammonia in the body to counteract this.

The acute lesions obtained by experiments upon animals do not differ essentially from those noted for man. Besides the various fatty changes and the ecchymoses already mentioned, Puppe found the fatty contents of the blood increased in a dog fed with phosphorus, and "free fatty crystals" may be seen. The same is noted by Menard, Wegner, and Corni and Ansiaux. Krönig after feeding phosphorus to a dog for thirty-six days found fatty degeneration of the liver, with dilatation of its vessels and bile ducts, and after seventy-nine days, hyaline and fatty degeneration of the hepatic vessels. After one hundred and six days the liver cells were vacuolated, their nuclei broken down or absent, foci of leucocytes and detritus of hepatic cells appeared, and increase of the periportal tissue. Lebedeff asserts that the fat in the liver is brought to the organ, and is hence a fatty infiltration, but his experiments are without value. Leo thinks that although the liver gains, and other parts of the body lose in fat, the total in the body is increased; consequently there must be some production. Leisering studied four fowls, two males and two females, accidentally poisoned by phosphorus, finding little beyond congestion in the organs of the former and marked fatty changes in the latter. Since the males were spare and the females were fat he argues that the fat was carried to the liver. But he reports that the kidneys in the females were fatty, and does not claim such a transport for these organs. Senftleben gave about 0.03 gram of phosphorus to a rabbit by injection, and on the following day about 0.015 gram by the mouth. Death occurred on the next day and already the myocardium was fatty and without striation, and there was beginning fatty degeneration in the villi of the intestine and in the kidneys. Wegner by careful feeding experiments with dogs, cats, and rabbits, carried on for several months, found the gastric mucosa swollen to as much as three times its normal thickness, indurated, and of a brown color, especially in the fundus, with elongation of the glands and increase of the stroma. The liver, beside the customary fatty changes, showed interstitial hepatitis "in optima forma." Silbermann injected phosphorus oil subcutaneously in dogs and obtained multiple autochthonous thrombi in various organs and peripherally. His opinion was that such thrombi might have a causal relation to the parenchymatous degenerations so characteristic of phosphorus poisoning. He also found marked hemolytic effects, the red cells becoming swollen and taking on various distorted forms, and the white cells becoming less numerous and more or less fragmented. Somewhat similar effects were observed by Fränkel and Rohmann in experiments on fowls. Taussig describes "an enormous destruction of the red cells" in fowls. Briliant found that both by injection and feeding experiments the blood pressure was reduced by phosphorus, but even more by PH_5. Falck states that subcutaneous injections of phosphorus in oil cause great increase in general metabolism in animals, with increase in the activity of the kidneys and the amount of the urine, decrease of the activity of the lungs and skin, and that from 54.4 to 97.6 per cent of the phosphorus injected was eliminated by the urine as P_2O_5. Taylor (Journ. Med. Research, Vol. IX., No. I., p. 59) finds that an essential action of phosphorus is its liberation of fat from combination with proteids, reversing "a normal metabolic relation." The result of such a proteolysis is the appearance of free fat in the cell.

EXPERIMENTAL.

In our experiments, dogs I. and II. were given the following doses of P_4S_3:

	Dog I.			Dog II.		
Day.	A.M.	P.M.	Daily total.	A.M.	P.M.	Daily total.
	Gram.	*Gram.*	*Gram.*	*Gram.*	*Gram.*	*Gram.*
1	0.218	0.000	0 218	0 217	0.000	0.217
2	0.046	0.040	0.086	0.042	0.043	0.085
3	0.051	0.063	0.114	0.053	0.045	0.098
4	0.045	0.064	0.109	0.048	0.057	0.105
5	0.052	0.065	0.117	0.048	0.075	0.123
6	0.030	0.023	0.053	0.053	0.067	0.120
7	0.097	0.135	0.232	0.136	0.154	0.290
8	0.142	0.135	0.277	0.149	0.112	0.261
Totals	0.681	0.525	1.206	0.746	0.553	1.299
Total, first period............			1.206	....		1.299

After the lapse of a week a second period of dosage began, as follows:

	Dog I.			Dog II.		
Day.	A.M.	P.M.	Daily total.	A.M.	P.M.	Daily total.
	Gram.	*Gram.*	*Gram.*	*Gram.*	*Gram.*	*Gram.*
1	0.128	0.085	0.213	0.106	0.090	0.196
2	0.092	0.093	0.185	0.084	0.098	0.182
3	0.073	0.078	0.151	0.086	0.064	0.150
4	0.076	0.073	0.149	0.134	0.082	0.216
5	0.055	0.067	0.122	0.060	0.059	0.119
6	0.096	0.099	0.195	0.096	0.107	0.203
7	0.118	0.092	0.210	0.068	0.100	0.168
8	0.096	0.097	0.193	0.101	0.109	0.210
Totals	0.734	0.684	1.418	0.735	0.709	1.444
Total for second period			1.418			1.444
Brought forward			1.206			1.299
Total amount received			2.624			2.743

Average daily dose, first period,	0.15075	0.1623
" " " second period,	0.1772	0.1805
" morning dose,	0.0884	0.0925
" evening "	0.0753	0.0788

Dog III. received single large daily doses for four days, as follows:

First day	1.000 gram.
Second day	1.516
Third day	1.600
Fourth day	1.845
Total	5.961
Average daily dose . . .	1.4902

Dog IV. received a single dose of 10.0 grams.

Each dog received the following daily food in two portions:

Meat	160 grams.
Crackers	40
Lard	30
Water	430 c.c.

At the beginning of the first period the weight of dog I. was 7,820 grams, and at the end 7,707, being a loss of one hundred and thirteen grams. Dog II. at first weighed 6.880 kilo and at the end 7.480, showing a gain of six hundred grams. The total daily amount of the urine and its specific gravity could not be determined, as the animals could not be catheterized. The cages were washed down with distilled water and the urine made up to a definite amount (500 c.c.), and aliquot quantities of this were taken for analysis.

The feces were dried to constant weight. Before medication the daily amount of feces was about eight grams in the moist condition. After administering the tetra-phosphorus tri-sulfid the daily amount of feces increased, averaging fifteen grams for the first period. In consistence the feces were fluid, and at times there was slight diarrhea. Dog I. lost a little blood from the bowel on one occasion; this was due to his having swallowed a bit of wire in his food. When the P_4S_8 was stopped, the daily amount decreased, but rose again during the second period. Dog II. suffered from diarrhea on stopping the drug. The attack lasted but a day and the amount of feces was fifty-five grams. The average amount was higher throughout the second period and the average daily dose was also higher. The curve of the daily totals of feces thus shows a steady upward movement.

METABOLISM OF NITROGEN. — The total nitrogen in the diet was 5.88 grams. In the case of dog I., for the two weeks preceding medication the amount of assimilated nitrogen approached the available total without actually reaching it except on the eighth; and the daily variations were all above the level of five grams except once, on the eleventh. It is therefore safe to say that of the available 5.88 grams of nitrogen only 0.88 gram were not assimilated. This continued for three days after beginning the administration of phosphorus tri-sulfid, until, in other words, the animal had received 0.418 gm. of the drug. From that point there was a distinct lessening of the nitrogen assimilated, a recovery at the end of the first period, and a second fall which lasted through the same period.

The corresponding curve for dog II. represents a similar fall during and after the first period, a recovery during the pause, and a lower average afterward. The fluctuations were marked after the end of the second period, reaching the lowest level of the whole series on the fifth day after discontinuing the doses.

The curve of nitrogen balance was kept for dogs I. and II. and showed practically a balance before medication, although the nitrogen was not totally assimilated. Then, with the beginning influence of the phosphorus compound, the slight apparent deficiency was exaggerated, and after stopping the drug the dogs had not nearly returned to a condition of nitrogen balance. In further experiments the animals will be kept to see when they return, if ever, to an equilibrium.

In the case of dog II. the amount of nitrogen in the feces was charted. At the end of each administration period there is a decided increase in the amount recovered. The curve persists on a high level for ten days after stopping the drug.

These variations in the assimilation of nitrogen may be explained in part by the lesion in the alimentary canal, which undoubtedly impaired the absorbing power of the mucous membrane, but the synchronous destruction of proteid in the formed elements of the body must also be taken into account.

The metabolism of phosphorus during the first period for dog I. rose steadily, as follows:

Dog I., Day 1	0.05 gram.
2	0.10
3	0.18

Dog I., Day 4	0.15 gram.
5	0.23
6	0.22
7	0.20
8	0.28
Second day of pause	0.38

From the latter point it fell through the remainder of the pause and the first days of the second period. In the case of dog II. the trend of the curve was upward, from 0.20 gram at the first to 0.44 gram on the second day of the pause. The observations for both dogs were untrustworthy for the second period, owing to inaccurate collection of the material, and are not given.

METABOLISM OF SULFUR. — For both dog I. and dog II. the fluctuations in the total sulfur contained in the urine during twenty-four hours were marked before the administration of P_4S_3, with a general upward trend for the ten days preceding medication. The highest point reached, from a start at 0.16 gram for both dogs, on the eighth, was 0.261 five days later for dog I., and 0.212 nine days later for dog II. In each period of treatment there was an evident fall, but in the first period this occurred during the beginning with each dog and in the second toward the end of the period. Between, there was a recovery, which was well marked at the end of the first period, lasting through the pause and the early half of the second period.

The general course of the curve for ethereal sulfur is upward, beginning with the first medication and rising steadily through both periods and the pause between. Dog I. shows the following figures, given in tenths of a gram, on alternate days through the whole twenty-two days:

Dog I., Day 1	2.3
2	1.0
4	4.0
5	1.8
7	3.4
9	2.3

Dog I., Day 11	3.4
13	3.0
16	4.8
18	8.3
21	10.1

A similar but less pronounced rise was evident in the curve of ethereal sulfur in the case of dog II. With this dog there was a fall after stopping the second period, the curve running down for the next ten days. On the whole, the variations in the excretion of ethereal sulphates were of more restricted range than those of alkaline sulphates, both actually and relatively.

METABOLISM OF FAT. — The disturbance in the metabolism of fat was even more marked than in the case of nitrogen. The feces often contained masses of undigested fat on gross examination. The curve of fat assimilated underwent great fluctuations, and the curve of unassimilated fat, excreted as such in the feces, rose steadily for dog I., and during the second period for dog II. On the whole, these characters were less evident for dog II. than for dog I. The daily record of fat in the feces of dog I. is as follows:

Dog I., Day 1	0.8 gram.
2	3.4
3	1.0
4	0.8
5	8.0
6	8.0
7	4.0
8	8.4
	Pause of one week.
9	3.0
10	3.2
11	1.2
12	1.2
13	3.6
14	1.0

	Second Period.
Dog I., Day 16	11.0
17	12.0
18	4.4
19	4.4
20	8.4

For dog II. the variations ran from 2.0 grams on the first day to 7.4 grams on the fifth day of the first period. After stopping the drug the tendency was to return, in each case, to the original low level, without ever quite reaching it.

It is interesting and important that neither albumin, sugar, leucin, nor tyrosin was ever detected in the urine of any of the dogs.

Sevéne and Blanc report that animals exposed to the vapor of tetra-phosphorus tri-sulfid succumb sooner or later. The fifth experiment consisted in covering a rabbit with a large bell jar, through which air was passed at the rate of five hundred cubic centimeters per minute. All the air thus passed through went first through five layers of the compound, alternating with layers of absorbent cotton, and then through two water bottles containing respectively solutions of acetate of lead and nitrate of silver. Before passing through the compound the air went through a wash bottle containing water, and was saturated with moisture. After several hours in this apparatus and under these conditions the rabbit showed absolutely no effect. The bottle containing the nitrate of silver was then removed, so that the respired air contained any volatile compounds of phosphorus which might be taken up. Nothing was noted beyond slight acceleration of the respiration. The animal was quiet and fed readily. The bottle containing acetate of lead was then removed, so that the animal breathed any volatile compounds of phosphorus or sulfur which might have been absorbed. The condition of the animal remained normal, and after these observations had been carried on over three days they were abandoned.

PATHOLOGICAL.

The dogs were killed by puncture of the medulla, except the fourth animal, and the organs were put in hardening fluids immediately. The records of the gross and microscopical examinations were as follows:

Necropsy of male dog No. 1., Dec. 10, 1901, weight 7.7 kilo.

Animal well nourished. General inspection of the abdominal contents discovers nothing abnormal.

Liver. Color pale, consistency normal, markings of acini distinct.

Kidneys. Capsules peel easily, surfaces smooth and pale, cortices pale, slightly swollen, bulge a little above the section, markings indistinct, glomeruli visible as minute red points.

Spleen. Normal in color, size, and consistence.

Pancreas. Pale and anemic.

Mesenteric nodes distinctly swollen and pink.

Stomach. Slight congestion in fundus, contains a small amount of undigested food, mucosa apparently normal.

Duodenum. Mucosa thickened and bile stained, surface of small intestine throughout covered with thick, frothy mucous; agminate and solitary follicles prominent, being slightly edematous; no fecal odor in any part of intestine.

Heart in valves and myocardium apparently normal.

The liver on chemical analysis contained 6.25 per cent of fat.

Microscopic Examination. — Liver. The cells in the peripheries of the acini are in a state of fatty degeneration, and yet, on the whole, even here the nuclei are well preserved and take the stain freely. In places the protoplasm is reduced to a mass of detritus and free nuclei. The bile ducts are normal. There is no excess of connective tissue.

Pancreas. The cells are everywhere swollen and the nuclei are vesicular; the protoplasm is slightly acidophile. The islands of Langerhans appear normal. The cells of the ducts are swollen, and this decreases their lumina appreciably.

Kidney. There is some active hyperemia of the cortex, but not of severe degree. The epithelia of the cortical tubes for the most part are well preserved, only here and there the nuclei are vesicular, and there is cell detritus in the lumina of the tubes. The Malpighian bodies and the looped tubes are normal.

Spleen. The organ is generally hyperemic and a few small hemorrhages are found. The lymphadenoid bodies are slightly hyperplastic.

Lung. In general the organ is hyperemic and edematous, but not to a marked degree. In some areas there is hemorrhage into the alveoli, and about these are small thrombi of red cells in the capillaries.

Small intestine. In the villi there is a slight active hyperemia, the investing epithelia are well preserved, and there is an apparent excess of beaker cells.

Mesenteric node. Both hyperemic and edematous.

Heart. The vessels of the myocardium are congested, the muscle fibers are opaque, and their striation is indistinct, but the nuclei are generally well defined and take the stain well.

Prostate. Normal.

Necropsy of male dog No. II., Dec. 21, 1901.

Dog well nourished; weight 7 48 kilo.; the omentum contains a large amount of fat.

Liver. Very much congested, but apparently normal in structure.

Kidneys. Large, capsule peels readily, the surface is smooth and of a mottled red color. On section the cortex is swollen and the markings are indistinct.

Spleen. The lymph-adenoid structures are very prominent, otherwise the organ is normal.

Pancreas. The organ is small and congested and there is an apparent increase in its stroma.

Stomach. Full of undigested food and the mucosa shows slight general (functional) hyperemia.

Small intestine. The mucosa is thickened and covered with an excess of mucus. In the lower portion there is some pigmentation with bile and the same excess of mucus. No fecal odor observed throughout the intestine.

Heart. Normal.

Microscopic Examination. — Liver. The protoplasm of the hepatic cells is coarsely granular or actually lacking in many places, but the nuclei persist and take the stain well. This is true of the entire acinus. The capillaries in the acini are hyperemic. There is no pigmentation of the organ. The biliary capillaries are pervious and their epithelia are normal.

Kidneys. There is acute arterial hyperemia throughout the cortex, and in its outer zone there is much granular degeneration of the epithelia. In the lower zone, just above the pyramids, and in the columns of Bertini, the tubes present either a granular epithelium whose nuclei are well preserved, or the cells are beginning to break down.

Spleen. The lymphoid tissue is copious and proliferating; elsewhere there is much granular brownish-yellow pigment, both free and enclosed in leucocytes.

Pancreas. The protoplasm of the cells is distinctly acidophile and finely granular; the nuclei are distinct and stain well, but are generally vesicular and have their chromatin disposed in minute granules; the nucleoli are large and prominent. The vessels are normal, there is no increase of connective tissue, and the epithelia of the ducts are slightly swollen but not granular.

Small intestine. The investing epithelium shows an excess of beaker cells. The core of each villus presents a dilated lymph channel; and the solitary follicles are large and their lymphatics are wide and contain dark amorphous pigment, both free and in leucocytes. The leucocytes of the follicles have scanty granular chromatin in their nuclei. Some villi are slightly hyperemic.

Heart. Striation of the muscular fibres well preserved, no fatty degeneration or hyperemia noted.

Adrenal. The protoplasm of the cells in the middle and outer zone is marked by fine vacuoles; in the central zone there is little vacuolation but some hyperemia.

No trace of hemolysis is observed in any of the vessels of the organs examined.

Necropsy of male dog, No. III , Dec. 5, 1901. Well nourished, weight 6.6 kilo.

Liver. The organ is large and congested, and the section is mottled by areas of fatty infiltration. Elsewhere the peripheries of the acini are light colored and clearly defined, marked here and there by minute yellow collections of fat. The gall bladder contains orange-yellow bile with small black grains of pigment.

Kidneys. Capsule strips easily, surface generally smooth, cortex swollen, and markings indistinct.

Spleen. Congested and marked by prominent lymphadenoid structures.

Pancreas. Large and pale.

Mesenteric nodes swollen and prominent.

Stomach. Contains undigested food and is slightly congested.

Small intestine. The mucosa throughout is swollen and covered by thick, frothy mucus, which is mingled with bile. There is no fecal odor. The solitary and agminate follicles are edematous.

Heart. Normal.

Prostate irregularly swollen and varies in color on section from light yellow to pink.

Microscopic Examination. — Liver. The central zones of the acini are hyperemic, the red cells being of perfect contour. The epithelia show marked granular degeneration, in places represented only by detritus, where remaining nuclei are large and have prominent nucleoli. There is but little pigmentation. The epithelia of the gall ducts are slightly swollen and their nuclei are large and vesicular.

Kidneys. There is active hyperemia of the cortex. The epithelia of the convoluted portions are swollen and in places block the lumina, in other places the granular protoplasm has broken down and left slender protoplasmic strands and bridges across the tube. In some tubes the epithelium is wholly desquamated from the wall. The glomeruli as a whole are shrunken, and in places there is a proliferation of the cells of the capsule of Bowman. The great collecting tubes contain cells, fatty epithelia, white cells, and granular detritus. The epithelia of Henle's loops are elongated and distorted. The coats of the arteries are thickened, especially the media.

Spleen. The cells of the Malpighian corpuscles are apparently hyperplastic and their nuclei are very large; otherwise the organ is hyperemic, and contains an excess of granular pigment, mostly extracellular.

Pancreas. There is marked eosinophilia of the central portions of the acini; the nuclei are large, and the nucleoli are prominent. The protoplasm of the cells is granular and in places vacuolated. The distinction between cell and cell is not always clear; they seem to be partly fused in places. The epithelia of the finer ducts are somewhat swollen.

Mesenteric node. Edematous, and the lining cells of the main lymphatic channels are swollen and project into the lumen.

Small intestine. The villi show many beaker cells among the investing cells, the core of the villus is edematous, and the lymph channel is dilated.

Stomach. The chief cells of the glandules are slightly swollen and granular. The superficial epithelia are well preserved and have a clear protoplasm.

Heart. Striation is partly lost, the fibers are a little swollen and their nuclei are irregular and distorted.

The liver on chemical analysis contained 4.14 per cent fat.

Necropsy of male dog, No IV., weight about 6.5 kilo. General nourishment good. Animal killed by section of great vessels of neck. General appearance of organs in consequence bloodless.

Liver. Pale, central vessels dilated, slight bile staining in places.

Kidneys. Normal in size, capsule strips imperfectly, surface pale and presents a few cysts. In section the cortex is slightly swollen and the glomeruli are visible.

Spleen. Slight hyperplasia of lymphadenoid elements.

Pancreas. Pale, but apparently normal.

Mesenteric nodes. A few of them are enlarged and edematous.

Stomach. Physiological hyperemia; contains undigested food.

Small intestine. Lymphoid structures throughout edematous and prominent. In the lower third the mucosa is congested and bile stained, and the colon presents a few very small submucous hemorrhages.

Microscopic Examination. — Liver and bile ducts normal, not more fat in the peripheries of the acini than normal, slight biliary pigmentation.

Kidneys. Cloudy swelling.

Spleen. Slight lymphoid hyperplasia and excess of amorphous pigment; otherwise normal.

Pancreas. Protoplasm generally intensely acidophile and markedly granular. Islands of Langerhans hyperemic. Epithelia of ducts swollen to partial occlusion of the lumen in places.

Mesenteric node hyperemic and edematous.

Stomach. Normal; lymphatics very wide.

Small intestine. Villi profusely infiltrated by mononuclear leucocytes.

Adrenal. Central zone hyperemic. Protoplasm generally finely vacuolated.

Testis. Normal.

It is evident from these gross and microscopic lesions that the action of tetra-phosphorus tri-sulfid is wholly unlike that of phosphorus, though the assertion of Courtois-Suffit, that it is "not injurious, no matter how large the dose," cannot be accepted as accurate. Its effect is that of a mild irritant upon the structures with which it comes in contact, like the intestinal villi, but its action is more severe after absorption through the intestinal wall. The reaction in the mesenteric nodes is a mild exudative inflammation. It may reach the heart through the lymph stream or by way of the portal and the hepatic veins, but when circulating through the vessels of this organ it may cause only a local hyperemia and a cloudy swelling. This may be contrasted with Senftleben's experiment with phosphorus, in which a rabbit received about 0.045 gram in forty-eight hours, died the next day, and showed already marked fatty degeneration of the heart.

Carried to the liver in large enough doses, or over a long enough period of dosage, the compound may cause the epithelia to break down. This occurs first at the periphery of the acinus, but may involve its whole extent; in that case the lesion is less marked about the central vein. Even when the protoplasm of the cells is much affected the nuclei persist and take the stain well. Probably but little is excreted by the liver, or else its nature is so modified that it causes no appreciable lesion of the biliary epithelium. The cells in the bile ducts were not so much affected in any case as the epithelia of the pancreatic ducts, and in the latter the change consisted only in swelling and slight granular alteration.

The organ which probably excretes the major portion of the drug is the kidney, and the severity of the effect is here most pronounced, but one must be struck with the fact, even here, that the enormous doses, compared with phosphorus, produce surprisingly little effect, and also that the nuclei persist for a time after the protoplasm of the cell has been lost.

The form in which the tetra-phosphorus tri-sulfid circulates in the blood is undetermined, but it is notable that it causes no hemolysis.

The relation between the lesions and the symptoms, including the records of metabolism, is sufficiently clear and needs but little explanation.

The increase in the daily amount of feces during administration, the decrease in the assimilation of nitrogen (dogs I. and II.), the increased N in the feces (dog II.), and the increase in the fat recovered from the feces, can all be explained by the hyperemia and increased production of mucus along the alimentary tract. The lesion was hardly severe enough to be considered more than a mild catarrhal inflammation. The steady rise in the assimilation of phosphorus shows that absorption from the intestine was still going on, and the fall in the nitrogen balance, together with the lesions in distant organs, as noted, confirm this idea. Some of the increase in excreted nitrogen is due to the protoplasmic destruction, but the proportion has not yet been determined.

It is an important fact that no one of the dogs died, though receiving doses which, if phosphorus, would certainly have been fatal in all four cases. As soon as the drug is stopped there is an immediate and buoyant recovery. Of the four dogs, possibly No. I. would not have recovered ultimately, the others probably would have done so. Assuming that the other three would have recovered, and calculating proportional doses for an adult human being, we have the following statement of the relative toxicity of the sulfur compound as compared with pure phosphorus:

Dog II.	received	2.624	grams	in 22 days	equivalent doses for a man weighing 60 kilo.	22.88 gram.
Dog III.	"	5.961	"	" 4 "		63.28 "
Dog IV.	"	10.	"	" 24 hours		90.9 "

Taking Kolisko's higher figure as the lethal dose of phosphorus, namely, 0.20 gram, it is evident that P_4S_3 is one hundred times less toxic than phosphorus on the least favorable showing, and over four hundred times less toxic according to the last experiment. In other words, no amount of tetra-phosphorus tri-sulfid, which any human being could take by accident or design, would probably prove fatal.

CONCLUSION.

1. Inhalation of air, passed through tetra-phosphorus tri-sulfid in substance, has no appreciable effect upon the rabbit at room temperature.
2. In the dog P_4S_3 acts as a mild local irritant upon the alimentary canal.
3. This action is more pronounced in the small intestine than in the stomach.
4. In the circulation it acts destructively upon the protoplasm of parenchymatous cells.
5. The effect varies from a strongly acidophile character in the protoplasm to its conversion into detritus.
6. The nuclei of the parenchymatous cells show a marked resistance, remaining and taking the stain even after the body of the cell has disappeared.
7. The effect is most marked in the epithelia of the renal cortex, of the liver, and of the pancreas, and in the muscular fibres of the heart, in the order named.
8. The destruction is manifested after long treatment with small doses or shorter periods of heavy dosage.
9. Perhaps the rapid recovery of the animals after stopping the administration is connected with the persistence of the nuclei.
10. In general the agent increases catabolism by this action upon epithelia, while at the same time lessening anabolism by its local and alimentary effects.
11. It does not cause hemolysis, nor jaundice, nor the appearance in the urine of albumin, sugar, tyrosin, nor leucin.

12. Consequently tetra-phosphorus tri-sulfid may be considered non-toxic in any ordinary circumstances.

(Note. — Our thanks are due to Mr. C. D. Partridge, B.Sc., for valuable assistance in the preliminary work connected with this investigation.)

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A CASE OF MULTIPLE FIBROMA (FIBRO–NEUROMA) OF THE NERVES OF THE LOWER EXTREMITIES, WITH DIFFUSE ENLARGEMENT OF THE SCIATICS — COMPLICATING SARCOMA AND METASTASES IN THE LUNGS.*

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I. INTRODUCTION.

Since the report of two cases of multiple neuroma by Cheseldon in 1740 and Tilesius in 1798, many studies of multiple fibromata of the nerves have been made. The most recent summary by Plebe and Hektoen contains an analysis of such cases, and to this we may refer.

Altogether when the reports are critically reviewed some seventy cases of these so-called multiple neuroma are on record.

The purpose of this paper is to record a new case in which the primary lesion is complicated by the occurrence of sarcoma. This occurrence of sarcoma in connection with multiple fibroma of the nerves has been recorded by Recklinghausen, Westphalin, and Garrè, and such cases form a group of considerable scientific interest and practical importance. Recklinghausen first drew attention to the relation of fibrous skin tumors to those of the nerves and their subsequent malignant transition; since then Garrè, after a review of the published cases, has collected seventeen of sarcoma of the nerves, which he has divided into two groups, viz.: first, a group characterized by multiple skin tumors, tumors of the peripheral nerves, pigmentation of skin, and functional disturbances; and second, a group in which the above conditions are absent; but in their stead we have enlargement alone of one or more nerves. Both groups may be complicated later by the development of a malignant type of growth and the formation of metatases.

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I shall not touch on the clinical history or the surgical aspects, because they will be made the subject of a contribution by Dr. Kammerer, to whom I am indebted for the case, except to state that the patient was a female, age 24, who entered St. Francis Hospital on May 9, 1901, with a large tumor on the posterior part of the right thigh, and with a similar but smaller tumor on the posterior part of the left thigh. She was operated upon May 23, 1901. Death occurred June 19, 1901.

II. AUTOPSY.

The autopsy on June 19, four hours post-mortem. Body is well preserved, but greatly emaciated. On the posterior part of the right thigh is a large, soft tumefaction extending nearly to the trochanter. A long incision resulting from the operation leads directly into a large cavity, the base of which is made by the shaft of the femur, and its walls by the very soft, spongy, and gelatinous muscles of the leg. There are large areas of blood infiltration mixed with gelatinous tumor mass extending up and down the thigh. The muscles of the posterior group are absent. The shaft of the femur shows no necrosis and the sciatic nerve cannot be found at this point. The nerves of the lumbo-sacral plexus on this side show marked enlargement from their exit, from the spinal canal to their junction to form the great sciatic — the diameter of each nerve being 1.5 cm. At the sacro-sciatic foramen the diameter of the sciatic nerve is 3.5 cm., while the distance from the foramen to where the nerve ends and is replaced by tumor mass is seventeen centimeters. From the point to the junction of the internal and external popliteal nerves, a distance of twenty centimeters, the great sciatic nerve is absent, its divided ends merging into the surrounding tumor tissue. The internal popliteal nerve is enlarged, its diameter being one and a half centimeters. From here down, the posterior tibial nerve is of about the same diameter till it divides into the internal and external plantar, the diameter of the latter being one centimeter. The anterior tibial nerve is one centimeter in diameter throughout. The anterior crural nerve and its branches show no enlargement. The nerves were of firm consistence and presented here and there nodular swellings. For the most part the great sciatic and its branches seemed to be of uniform thickness throughout. The small cutaneous nerves were thickened and presented many nodular swellings along their course, giving them a beaded appearance. This latter condition was especially well marked in the small sciatic and superior gluteal, where the nodular swellings connected with these nerves were so abundant as to make a lobulated mass above the tuberosity of the ischium.

The dissection of the left sciatic nerve discloses a somewhat different condition to that of the right, in that the continuity of this nerve is preserved, although presenting a tumor of much smaller size within the nerve sheath. The nerves of the lumbo-sacral cords at their exit from the spinal canal are 1.5 cm. in diameter; and at their junction to make the great sciatic nerve at the foramen is three centimeters in diameter. From here down, the nerve is of a uniform thickness to a point twenty-five centimeters from the sacro-sciatic foramen where it enlarges into a fusiform swelling which is ten centimeters in longitudinal direction six centimeters in diameter, the whole tumor being fourteen centimeters in circumference. The longitudinal section of this fusiform mass discloses a tissue of moderate consistence, irregularly mottled with light yellow and grayish gelatinous areas and encapsulated by the nerve sheath; near the lower part there is commencing cyst formation. The sciatic nerve entering this tumor at its upper pole forms a very delicate plexus at its periphery, when the nerve is again restored at its lower end into its appropriate branches. From this point down the internal popliteal, posterior tibial, plantar, and anterior tibial nerves are enlarged, their diameters being the same as those of the right leg. The small cutaneous nerves are thickened and present many nodular swellings along their course. The brain and spinal cord are normal. On the branches of the cauda equina are a few fusiform swellings. The dissection of the epigastric, sympathetic, cervical, and brachial plexuses show no changes or enlargement of their branches.

The heart, liver, kidneys, spleen, and gastro-intestinal tract present no abnormal conditions. In both lungs, however, are numerous tumors, varying in size from a hazelnut to a hen's egg, of grayish color and gelatinous consistence. These are scattered irregularly through the lung parenchyma. Visceral and parietal pleura are normal. In the diaphragm on the right side are several embedded masses of firm consistence and somewhat sharply circumscribed in the diaphragmatic tissue.

In the head of the pancreas are three large nodules of a firm consistence, the rest of the organ being normal.

III. MICROSCOPICAL EXAMINATION.

Portions of the tumor and thickened nerves were hardened in formalin, ten per cent, Orth's fluid, Flemming's solution, and alcohol, and stained by Weigert-Pal, hematoxylin and eosin, Van Gieson and Weigert's elastic tissue methods.

The changes in the heart, liver, kidneys, and spleen were those of parenchymatous degeneration.

(*a.*) Sections of the right sciatic nerve, three centimeters below the foramen, show uniform enlargement and give a picture of diffuse sclerosis. The connective tissue of the perineuron running in between the nerve bundles in narrow and wide bands divides the nerve into smaller and larger compartments. There is marked increase of fibrous tissue of the epineurion, but more so of the endoneurion. The number of nerve fibers is notably diminished and replaced by connective tissue of great density. In some bundles only a few fibers are to be found, while a surprisingly large number of well-preserved fibers could be found in others. Sections stained by Weigert-Pal and Van Gieson methods show well the loss of nerve fibers and increase of connective tissue. The individual nerve fibers, although in general compromised by the new-formed fibrous tissue, are surprisingly free in many sections of the enlarged popliteal and anterior tibial nerves. Sections of the smaller and larger tumors connected with the peripheral nerves present the characteristic structure of loose texture fibroma. The fibrous tumors appear to spring from the nerve sheath which sends in bands of denser connective tissue. These nodes consist of typical fibrillated connective tissue which is extremely tangled and separated by a fluid stroma containing many medium sized degenerated cells. The number of blood vessels varies greatly in many of the nodules. Sections stained by Weigert-Pal method show no nerve tissue in the nerve itself, but generally a few fibers can be seen at the extreme periphery of the sheath.

Sections of Large Tumor which had been partially removed from Right Sciatic Nerve.—The cells of this tissue are of the polyhedral type and vary in size; in places they are packed together with fibrillated stroma between, while in other areas separated by a fluid stroma staining by hematoxylin in which are many fine fibrils staining especially well by Flemming's hardened tissue. The blood vessels are numerous and for the most part thin-walled, showing in places cellular proliferation at their periphery. Mitotic figures are abundant and areas of necrosis at the center of the tumor quite large. The tissue has all the characteristics of a typical sarcoma. Sections of the tumor stained by Weigert-Pal show no nerve fibers.

(*b.*) Sections of left sciatic nerve and its branches. As the microscopical picture of the left sciatic nerve beyond the tumor presents an identical appearance to that of the right sciatic nerve and its branches, which have already been described, it will be unnecessary to repeat. The tumor of this left sciatic nerve, being, however, structurally different, will be here described.

Sections from this tumor are chiefly made up of spindle cells with an abundant intercellular fibrous stroma. In places the stroma is dense with few cells, while in other areas it is very cellular and edematous. The cells run in various directions, but seem to have a more or less close relation to the adventitia of the blood vessels. The nuclei and cells vary considerably in size and shape. Many huge, deeply stained chromatin masses with a proportionate amount of protoplasm are by no means uncommon. The tissue is well supplied with thin-walled blood vessels which seem to be intimately associated with the tumor cells.

Sections of Metastatic Tumors in Lungs. — Sections from these nodules present an identical appearance to the large tumor of right sciatic nerve; viz., they are made up of polyhedral cells, intermingled with fibrillated stroma, and contain blood vessels. It is perfectly evident that histologically these tumors are the direct result of metastatic invasion from the right sciatic nerve tumor.

Sections from Tumor Masses in Pancreas and Diaphragm. — Sections from both tumors present an identical appearance. They are surrounded by a fibrous capsule, the tumor elements composed of spindle cells intermingled with stroma and blood vessels. I do not think that these tumors have any connection with the tumors described on the sciatic nerves, although histologically they appear like the tumor of the left sciatic nerve. I am inclined to class them as isolated examples of malignant transitions in the thickened nerves supplying the pancreas and diaphragm, viz., those nerves of the splenic and phrenic plexus.

Anatomical Diagnosis. — Multiple fibromata of both sciatic nerves and branches — complicating sarcoma — metastases in lungs.

The most important features of this case, then, are that it adds another to the list of neuro-fibromatoses of the nerves described by other writers, that it is of especial interest in showing the bilateral condition of tumor growth on the sciatic nerves, and also that it is an admirable example of that class of cases described by Garrè; viz., that there exists a congenital predisposition for nerves to undergo diffuse sclerosis, to sarcomatous metaplasia, and the formation of metastases.

A CASE OF SEPTICEMIC GLANDERS IN THE HUMAN SUBJECT.[1]

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The comparative rarity of the occurrence, or at least of the recognition of glanders in the human subject, and the somewhat remarkable features of this case, seem to warrant the detailed report of the study of a case of glanders examined by the writers in September, 1901. This case is the fourth, according to the records of the Health Department, that has occurred in New York City since 1888, viz., one in 1888, one in 1893, and one in 1899; but Park failed to obtain the glanders bacillus from the material of the third case, and believes that it was really not glanders.

CLINICAL HISTORY.

John E. D., aged 36 years, hostler, and occasional "veterinary surgeon" by occupation, was admitted to Bellevue Hospital, Aug. 24, 1901. Three weeks previously the patient had been bitten by a horse, said to have "distemper," on the third finger of the left hand. The wound suppurated, was treated by a physician, and healed after ten days. The patient had no constitutional symptoms until the wound had healed, or about ten days before admission. So far as could be elicited from him or his friends, there had been no involvement of the lymph nodes of the arm.

The illness began with headache, disinclination to exertion, vague muscular pains in the back and legs, loss of appetite, and diarrhea. There was neither chill, epistaxis, nor cough. The patient grew progressively weaker, and sought admission to the hospital.

August 24th.— Physical Examination by Dr. G. C. Buck.— The patient is well nourished and muscular. The face is flushed and apathetic; the eyes are suffused and dull. The tongue is coated over the centre, red at the tip and edges, and markedly tremulous. The pulse is 88, regular, of good force, and compressible; the temperature is 104.6°.

The heart is normal in size, the sounds are of fair quality and unaccompanied by murmurs. There is no abdominal distention or tenderness. A few rose-colored spots, somewhat similar to those of typhoid fever, are

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present over the upper part of the abdomen. The liver is normal in size. The spleen is not palpable, but the area of splenic dullness is slightly enlarged. The wound of the finger is completely healed.

August 25th.— The patient is more apathetic, and at times slightly delirious. A tender fluctuating swelling has developed in the middle outer anterior aspect of the left leg. The overlying skin is red. The "rose spots" on the abdomen are more numerous, and some are distinctly raised above the surface.

August 26th.— The patient is very talkative, and at times actively delirious. The swelling on the left leg has grown larger. About 6 oz. of thick, grayish, blood-streaked pus is discharged after incision. Some of the "rose spots" on the abdomen have become distinctly papular, and a few small papules have appeared on the forehead, face, chest, and back. A swelling which does not fluctuate has developed in the middle third of the left forearm. The leucocyte count is 13,000.

August 27th.— The general condition of the patient is unchanged. There is very little discharge from the abscess of the left leg. The swelling in the left forearm fluctuates. About 4 oz. of pus is evacuated by incision. Microscopic examination of the pus shows cocci only. A few of the papules previously noted have become pustular. New papules have appeared in the same regions. About this time the patient began to emaciate rapidly.

August 28th.— The patient is delirious most of the time and is decidedly weaker. There is almost no discharge from the abscesses. Several new abscesses are present to-day — one in the right forearm, fluctuates, overlying skin normal; one in the outer part of left thigh, three inches above the knee-joint, does not fluctuate; one in the outer middle third of the right leg fluctuates and is red and tender. The patient has developed a short, hacking, non-productive cough. Widal's reaction is negative.

August 29th.— The delirium continues. The patient is oblivious to all that happens around him. Some of the pustules have been ruptured by the nurse in washing the patient, and are excavated. Some appear umbilicated.

August 30th. The patient's general condition is much worse. Numerous subcrepitant and mucous räles are heard over both lungs. The breath sounds are roughened (the patient talks constantly, preventing a satisfactory examination). The papules and pustules are much more numerous. About 3 oz. of thin grayish pus is obtained from the abscess of the right forearm. The leucocyte count is again 13,000.

August 31st.— The patient is very weak, and has a low, muttering delirium. The eruption is more abundant. Smear preparations made from the pustules contained cocci and a few bacilli possessing the morphological characters of *B. mallei.* Cultures were made from several pustules and an abscess, and 5 c.c. of blood was taken from a vein at the bend of the elbow for bacteriological examination.

September 1st.— Death occurred at 7.45 A.M.

REMARKS ON THE CLINICAL HISTORY.

The abscesses which developed in the extremities showed a remarkably symmetrical distribution. All of them were deeply seated, and for the most part without signs of inflammation in the overlying skin. The pus flowed readily from the abscess cavities, was of a dirty grayish color, and often blood-streaked. It contained very few organisms, and at the first examination only cocci were found. The abscess cavities did not have a pyogenic membrane. The pus formed within the muscles, but ruptured through them and spread along their sheaths for variable distances. The abscess in the lower part of the left thigh had burrowed nearly into the knee-joint. Practically no reaction took place in the incision wounds. The abscess cavities remained nearly dry.

The eruption appeared about the eighth or ninth day of the disease as a few macules scattered over the anterior abdominal wall. They resembled in some respects the "rose spots" of typhoid fever. It was demonstrated that some of the early macules disappeared without reaching the papular stage, as several, identified by surrounding them with an indelible pencilmark, had left no trace at subsequent examination. By far the majority of them developed into papules which subsequently became pustular. The eruption came out slowly at first, then with great suddenness, almost over night numerous papules and pustules appeared on the forehead, face, chest, and upper part of the abdomen. While the eruption was chiefly confined to these situations, scattered lesions were found over the scalp, arms, and back. During the early part of the disease, the change from macule to papule and pustule proceeded slowly, occupying two or three days, but later the transition was measured by hours. The individual lesions were acuminate papules or pustules situated on a hyperemic base, which became anemic after death. Woodhead, in Allbutt's system, states that the base is sometimes anemic during life.

Many of the lesions ruptured spontaneously or from traumatism and were then umbilicated, but during life and after death many of the unruptured pustules were distinctly umbilicated. The majority of the small lesions, however, were acuminate. The individual lesions resembled the pustules of variola, but were less numerous, most of them were acuminate, and their comparative scarcity on the face left no ground for confusing the disease with variola. Most of the lesions were discrete, but in several localities there was more diffuse involvement of the derma where the papules and pustules were conglomerate, and areas from two to four centimeters in diameter were indurated, swollen, and reddened. Some of these would undoubtedly have ruptured, leaving deep ragged ulcers, had the patient lived longer. (Fig. 2.) The nasal mucous membrane gave no evidence of involvement during life.

The cough was due to lesions of the trachea, bronchi, and lungs, which will be fully described in the pathological report. The rate of respiration became accelerated during the last three days of the illness, reaching forty per minute. None of the accessible lymph nodes were swollen.

The range of temperature was high. Only once did it fall below 103° F. to 102.8° F., and the highest point reached was 105.6° F.

Until within two days of death the pulse rate was remarkably low, when one considers the temperature curve, varying from seventy-eight to one hundred per minute.

The urine examined the day after admission was pale, acid, of 1,005 specific gravity, and contained neither albumin nor sugar. The microscopic examination was negative. The urine was not examined again.

There was slight diarrhea during the course of the disease.

The treatment was confined entirely to supporting and hydrotherapeutic measures. As the attempts to reduce the patient's temperature by cold baths were futile, and as they seemed to distress him, they were discontinued.

The diagnosis of acute glanders was not made without difficulty. The high temperature, the disproportionately low pulse rate, the spots, resembling as they did at first the rose spots of typhoid, the tremulous coated tongue, together with the season of the year, naturally suggested typhoid fever. But the day after admission, when the abscess of the leg had appeared and some of the "rose spots" had changed to papules, the idea of typhoid fever had to be abandoned. The pus from an abscess was examined for whatever organisms might be present and only cocci found. As fresh abscesses appeared and as the eruption became pustular, the history of the horse-bite took on greater significance, and the pus was searched anew for bacilli.

At this second examination a few bacilli were found presenting the morphological characters of B. mallei, and pending the result of the bacteriological examination of the blood a provisional diagnosis of acute glanders was made. The post-mortem findings and the cultures from the blood subsequently confirmed this diagnosis.

The incubation period. — Woodhead states that the incubation period after inoculation is very short, twenty-four hours, unless the inoculation takes place through an old wound or the infection is cryptogenic, when the period may be prolonged to one or two weeks or more. Moreover, after inoculation involvement of lymph nodes is prominent.

If these rules apply to the present case, infection could not have occurred when the wound of the finger was received. It may have occurred through this wound during its healing, but this appears unlikely as the wound was treated aseptically and healed after some suppuration. It is still less probable that the infection was of cryptogenic origin, and considerable doubt has been thrown upon the genuineness of some reported infections of this type. In view of the clear history of a bite from a sick horse said to have "distemper," and of a pyogenic infection of this wound, it would seem to us most reasonable to conclude that the glanders infection occurred when the finger was bitten or shortly thereafter, and that dissemination of the bacilli occurred much more slowly than is indicated in the rules laid down by Woodhead. In Duval's case the outbreak of the disease in acute form occurred fifteen days after a wound of the finger.

PATHOLOGICAL REPORT.

Autopsy thirty hours after death.

The *skin* exhibited several hundred papules, macules, and pustules, irregularly distributed over the body, varying in size from a pin head to 1 cm. in diameter. They were generally acuminate, sometimes slightly umbilicated, while the largest lesions were ulcerated and excavated. Many of them resembled variolous pustules, but were less distinctly purulent and not markedly umbilicated. They were surrounded by a peculiar anemic halo ½ cm. in diameter. In some areas the lesions were confluent.

The *skeletal muscles* were the seat of about a score of abscesses ½ to 3 cm. in diameter, containing thick blood-stained pus.

The *heart* was normal.

Both *lungs* exhibited numerous foci of consolidation, resembling small and large tubercles, single or more often conglomerate. These nodules projected slightly on section and on the pleural surface. They were quite firm, and were surrounded by areas of hemorrhage. Numerous larger consolidated foci were present in both lungs. The upper part of the left lung was slightly consolidated and of light yellowish-gray color. Here the pleura was coated with fibrin. The bronchial nodes were very hyperemic. Two of them were necrotic and softened, and one of these had ruptured into the left bronchus, producing a large ragged ulcer of the mucosa. The pneumonic area had apparently resulted from the aspiration of the contents of this node. The bronchial mucosa was very hyperemic, and presented a few prominent papules and pustules.

The *lymph nodes* of the left axilla, groin, and arm were not palpable, and were not dissected out; and no other lymph nodes were found to contain specific lesions.

The *spleen* was the seat of well-marked acute hyperplastic splenitis.

Liver and kidneys showed acute degeneration.

Cranium. Over the right side of the occipital protuberance was a subperiosteal collection of thick pus, and between the dura and skull a similar collection. The meninges were hyperemic and edematous, but the brain was normal.

The gastro-intestinal tract showed slight catarrhal inflammation. Peyer's patches were atrophic.

Bone marrow cellular and hyperemic.

Testicles and bladder normal.

There is a healed wound with irregular hyperemic scar on the tip of the left third finger.

Anatomical Diagnosis. — Pustular eruption of the skin and bronchial mucosa. Multiple intra-muscular abscesses. Necrotic bronchial lymphadenitis; rupture into the bronchus. Multiple granulomata and diffuse pneumonia of the lungs. Glanders.

MICROSCOPICAL EXAMINATION.

The pulmonary lesion. Throughout the lungs there is marked congestion, edema, and considerable catarrhal desquamation of epithelium.

The glanders nodules consist of a series of vesicles filled with exudate and surrounded usually by a zone of hemorrhage. The exudate consists of about equal proportions of mononuclear and polynuclear leucocytes, of exfoliated epithelium, and a considerable admixture of red cells. Fibrin is abundant in many places, and some vessels are filled exclusively with fibrin masses, but in the more cellular portions fibrin is absent. The exuded cells exhibit various degenerative changes, including karyorhexis, karyolysis, hydrops of nuclei and cytoplasm, and fatty degeneration. No collections of fat, such as described by some writers, could be detected.

A great many fusion giant cells are present, of many sizes, and exhibiting a variety of degenerative nuclear changes. The appearance of this exudate and especially of the giant cells is very characteristic. (See Fig. 2.)

It shows some resemblance to acute necrotic tuberculosis, but the giant cells are very different from those of tuberculosis, the necrosis is nowhere complete, and karyorhexis is much more prominent. Any tendency of the glanders bacillus to induce a productive lesion is entirely obscured by the intense exudative process.

The bronchi are the seat of an intense purulent catarrh.

The cutaneous lesion. The inflammatory foci are located in the derma close beneath the epithelium, or very often deeper near the subcutaneous fat. The larger lesions are conglomerate nodules composed of several small foci in which the exudate has gathered in a small cavity, marked off by the distended connective tissue fibres. The exuded cells appear to be principally mononuclear leucocytes, with fewer polynuclear cells, but the necrotic process is too far advanced to allow accurate identification of the cells.

There are some of the same fusion giant cells described in the lung. Karyorhexis is very advanced, very few cells are left with intact nuclei, and the whole mass of exudate exhibits very numerous pyknotic or hydropic nuclear fragments yielding a very characteristic appearance.

The necrotic area is surrounded by a zone of vessels showing great abundance of fibrin.

In the exudate the red cells have entirely disappeared. In the zone of vessels with excess of fibrin hemolysis has occurred, leaving only shadows, while on the outskirts of the lesion many small vessels exhibit thrombi of agglutinated red cells of homogeneous lustre and often forming the central points of fibrinous coagula.

The anemic zone surrounding the pustule appears to be referable, at least in part, to the numerous small dilatations of the sub-epithelial lymphatics which have raised the epithelium to such an extent as to readily explain its whitening.

The intra-muscular abscesses. (Fig. 1.) The process in the muscles is very similar to that in the lung and skin. The necrotic exudate is identical in appearance with that of the skin lesions. The advance of the destructive process is everywhere marked by a narrow zone of hemorrhage and edema. Later comes the cellular exudate of the usual type. The muscle fibres undergo a series of changes ending in their complete destruction. These consist in swelling, obliteration of Cohnheim's fields, invasion by leucocytes, vacuolation, and fragmentation. Many fibres dissolve without invasion by leucocytes, leaving a faint unstained network as if from complete hydrolysis. Half dissolved or completely dissolved fibres are sometimes seen a considerable distance from the necrotic zone. No trace of reparative proliferation of fixed cells could be discovered. The edges of the necrotic foci are often sharply marked off from very slightly altered muscle fibres. The red cells at a distance from the abscesses often show peculiar agglomeration, but no distinct agglutination, although some solution of Hb has occurred and many crystals of hematoidin are everywhere present.

The marrow. The chief characteristic of the bone marrow, as found in the ribs and vertebræ, is an extreme congestion of the vessels. The sinuses are gorged with blood, many are distended much beyond their natural limits and some are ruptured, giving rise to minute extravasations. The pulp cords are, as a rule, not more cellular than normal, but in certain scanty and narrow foci there is some increase in large neutrophile myelocytes. The lymphocytes also appear in normal numbers, and islands of nucleated red cells are numerous. A marked excess of fibrin is found in many small vessels — with this exception, the appearances are identical with those seen in the marrow of typhoid fever. No distinct hematomata as described by Ferrari and Guarnieri were found, but the bones of the extremities were not examined.

BACTERIOLOGICAL REPORT.

(*a.*) Circulating blood. On August 31, twenty-six hours before death, five cubic centimeters of blood were aspirated from the median basilic vein, and kept on ice until September second. The blood was then divided between two tubes of ordinary broth. After twenty-four hours in the thermostat no growth was visible, but on the morning of September fourth (forty-eight hours) both tubes exhibited a rather profuse growth, resembling that of streptococcus pyogenes, and consisting of opaque, whitish flocculi which adhered to the sides of the tube and to each other, leaving the fluid clear. The growth consisted of micro-organisms similar to Bacillus mallei and in pure culture. Cultures on blood serum of the blood post-mortem gave colonies of the glanders bacillus and of various unidentified cocci.

(*b.*) Pustules. On August twenty-ninth smears from the pustules contained a few staphylococci and a few bacilli resembling B. mallei. Post-mortem, from a pustule of the skin previously pencilled off with five per cent formalin and sterile water, a growth was obtained of the glanders bacillus and of a large unidentified coccus, while the smears contained only bacilli.

(*c*) Intra-muscular abscesses. The smears of the pus contained bacilli and cocci, and cultures gave a growth of B. mallei and staphylococcus aureus.

(*d.*) Lung. The sputum examined during life was found to contain many bacilli resembling B. mallei.

Smears of the lung contained cocci, bacilli resembling B. mallei, and a large filiform bacillus. Cultures gave Bacillus mallei in profuse growth, and a large filiform bacillus. The earliest identification of B. mallei was obtained from a culture of the lung which gave a diffuse brownish growth on potato, at first pure, later overgrown by the large filiform bacillus. This growth was obtained in less than twenty-four hours. In some serum tubes inoculated from the lung, Staphylococcus pyogenes aureus grew.

The bacteriological examination therefore gave a pure culture of B. mallei from the circulating blood during life and the morphological demonstration of this micro-organism in the sputum and pus from the abscesses; while after death the same bacillus was obtained from the same sources, but combined always with Staphylococcus pyogenes aureus, and in the lung with a large filiform bacillus. As the staphylococcus was not found in the circulating blood during life, but only in the post-mortem material, and as this micro-organism is so frequently present in the lung post-mortem, we are not inclined to attach much importance to its demonstration in any of the localities except in the abscesses where there seems to have been an active mixed infection.

In a case reported by Duval and others the glanders bacillus was obtained in pure but scanty culture from the circulating blood during life, but the writers can find no other reports of the bacteriological examination of the circulating blood.

Distribution of B. mallei in stained sections. Although the glanders bacillus is demonstrated with some difficulty in sections of tissue stained by ordinary methods, no great trouble was experienced in identifying this micro-organism in thin paraffin sections of the formalin-hardened tissues stained twenty-four hours in saturated watery solution of Ehrlich's rectified methylene blue.

In such preparations the bacillus could be identified especially by the pale staining spots which give it a somewhat serrated appearance. The bacillus was present in large numbers in all the lesions. Most of them were contained in medium-sized and large mononuclear cells and in the giant cells, in all of which cells they lay singly or in groups, often within distinct vacuoles. Some well-preserved cells contained large numbers of bacilli which distended the cell body. In the outskirts of the nodules where the cells were well preserved, nearly all the bacilli were intra-cellular, but in the necrosing centres many were found extra-cellular.

In sections of the skin the bacilli were present in moderate numbers, and none but those resembling B. mallei could be detected. In the abscesses and in the testicle staphylococci also were detected, while in the lung the glanders bacillus was considerably overgrown with cocci and filiform bacilli which latter were usually extra-cellular.

Some alteration in the intra-vitam numbers and position of the micro-organisms in the tissues must be granted to have occurred after death.

Experimental glanders in the guinea-pig. The usual positive results of inoculation of a male guinea-pig were obtained.

This pig was inoculated intraperitoneally with an emulsion of lung tissue on September third. It did not appear to be much affected till September eighth, when it rather suddenly collaped and died September ninth. The testicles were painful and partially fixed by the end of the third day, and later the scrotum became moderately swollen and edematous.

The autopsy showed a few small collections of caseous material in the skin and muscles at the point of puncture, while on the peritoneal surface adjoining were many fine granules. The visceral peritoneum was shiny. Ascites absent. There was one large pustule projecting prominently from the peritoneal surface of the small intestine. The omentum was shrivelled up into a globular mass one centimeter in diameter and exhibiting several small glanders nodules. Kidneys negative. Lungs, a good many minute grayish foci could be distinguished on section. The Malpighian bodies of the spleen were over-prominent. The liver showed numerous minute whitish foci on section.

The testicles were much swollen, together measuring four centimeters across. They were adherent to the scrotum, the cavity of the tunica vaginalis here being obliterated by a fusion of its layers from the inflammatory process. The lesions involved the entire surface of both testicles and epididymi and extended one centimeter up the cord. Sometimes only one testicle is involved. (Frothingham.) The neighboring parietal peritoneum of the abdominal cavity exhibited a score of fine superficial glanders nodules.

Microscopically the lesions of the testicles were found to have originated on the peritoneal surfaces which were fused together, while the process had spread through the muscular tissue of the scrotum and very often into the substance of the testicle.

In the epididymus the lesions were more extensive and had reached a suppurative grade with the production of small abscesses. Elsewhere the lesions were of the more typical type described in the human tissues. In the lungs and liver the whitish foci proved to be minute glanders nodules. In the liver there were innumerable nodules, the smallest involving but one or two cords of cells in which the liver cells and a few leucocytes were necrotic, nuclei subdivided and pyknotic. No mitosis could be detected. These nodules were identical in appearance with those described by Wright. Many lay alongside the small hepatic veins. Glanders bacilli in intra-cellular groups and fewer extra-cellular individuals were abundant. In the lungs there were the same evidences of focal necrosis about groups of bacilli, as well as some larger nodules.

Cultures of the heart's blood remained sterile.

Cultures from the testicles gave on broth Staphylococcus pyogenes aureus, on blood serum and potato B. mallei and Staphylococcus pyogenes aureus. The growth on potato was of typical brownish color and not whitish as seen with Kutscher's bacillus. Later a greenish shimmer appeared on the edges of the growth. The suppurative character of the lesions about the epididymus may perhaps be referred to the presence of the Staphylococcus pyogenes aureus.

EPICRITICAL.

In the literature on glanders there has always been diversity of statement and opinion regarding many features and even regarding the essential nature of the process. The observations in the present case, although not sufficiently extensive to warrant positive conclusions concerning all forms of the disease, yet have some bearing on the discussion of the disease in its peracute type.

I. Morphology and the distribution of the bacilli in the tissues. An extreme variety in the morphology of Bacillus mallei in cultures has been demonstrated by many observers, especially by Valerio. These described forms include, beside the usual short rods with pale staining segments, enormously elongated threads, chains, polar swelling, large uniformly swollen rods, extremely minute rods, and branching forms. Most of these irregular types appear to be limited to old cultures, but some have been described in tissues. These characters have necessarily led to the classification of Bacillus mallei as a streptothrix. These characters are perhaps most apparent in the tendency of the micro-organism to form large zoöglea masses, "*drusen*," in the tissues, similar to the colonies of actinomyces, as described by Loeffler, Mayer, and others.

In the present case most of the streptothrical characters were entirely absent. In the sputum, pus, and in the early cultures, practically the only form seen was a short bacillus, with pale stained areas, sometimes slightly swollen at one or both ends, sometimes moderately elongated. Here there was not much greater variation in morphology than is to be seen in the tubercle bacillus in the ordinary case of acute tuberculosis.

The early growth on potato was especially uniform in morphology, the bacillus appearing as a very short rod, usually single. Later these forms were replaced by longer ones and some threads, but the great variety of types described by Valerio did not appear. On blood serum the usual forms were striated rods, polar swelling, forms resembling chain cocci, and some resembling much swollen cocci. No attempt was made to follow the changes in morphology further, as our cultures seemed to show a tendency to remain uniform.

In the tissues the bacilli maintained the same general uniformity, the striated rod being the predominant form. In the skin and abscesses, however, elongated rods or threads or chains were sometimes encountered and many swollen forms resembling cocci were seen.

Although often seen in densely packed groups within epithelioid and giant cells, the micro-organism exhibited no tendency whatever to form large colonies as described by Mayer in experimental glanders, and occurring as the rule, in actinomycosis.

All of these observations accord with the conclusion of Conradi, that pleomorphism is rather strictly limited to the saprophytic existence of the bacillus, and that when growing as a parasite in the animal organism its forms are limited, many phases of development are omitted, and chain and branching forms never appear.

II. The nature of the glanders process. There are at present three main contentions regarding the essential nature of the inflammatory process excited by the bacillus mallei.

(*a.*) The essential feature of the glanders process is a proliferation of fixed tissue cells, with the formation of epithelioid cells and followed by necrosis and exudation. This view is held by Baumgarten, Loeffler, Lubarsch, Strube, and others. Virchow regarded the new cells as proliferated fibroblasts, but placed the lesion midway between the strictly productive process and purulent inflammation.

(*b.*) The first event after the lodgement of the bacillus in a tissue is the exudation of round cells (lymphocytes) followed later, and only on the outskirts of the lesion, by a growth of epithelioid fibroblasts. This view is held by the French school, Leclainche and Montane, Lecredde, and by Bollinger.

(*c.*) The first effect of Bacillus mallei on the tissues is a necrosis of tissue cells, followed by exudation of mononuclear and polynuclear cells, and often of blood and fibrin. This opinion is maintained by Tedeschi, Csokor, and Wright.

From a study of the literature on the histology of the lesions of glanders it seems evident that many observers have attempted to draw too rigid conclusions from the material under observation, while more or less completely ignoring the definite observations of others.

Thus one finds attributed to Baumgarten and many other astute observers the statement that giant cells do not develop in the glanders nodules. They were, however, a prominent and characteristic feature of the lesion in the present case. Unna states that no diapedesis of red cells occurs, which was rather strictly true of the cutaneous lesions in the present case, while elsewhere widespread extravasations of blood were present. Ferrari states that in the blood the bacilli are always found within the leucocytes, which can hardly be granted for a violent form of septicemia, and did not prove to be true in sections of blood in the present case. Many observers state that in the tissues the bacilli are mostly extra-cellular, and many others encountered material in which, as in the present case, the intra-cellular grouping of the bacilli was prominently noted. It appears that the course of the inflammatory process in glanders must vary a great deal according to the virulence of the infection, the susceptibility of the organism, and the structure of the tissue involved, and that Bacillus mallei may prove a strict tissue parasite or may actively invade the blood, that hemorrhage occurs in some cases and some tissues and not in others, that giant cells may be absent, that the location of the micro-organisms may be intra- or extra-cellular.

Regarding the essential nature of the inflammatory reaction excited by B. mallei the same variations seem to occur, and to indicate that the reaction may be principally and originally necrotic, or exudative and necrotic, or productive and necrotic.

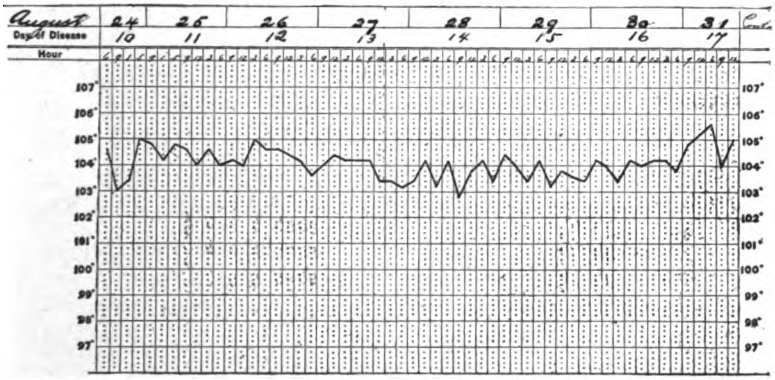
It appears that many of these variations may be controlled at will. When one rubs up a killed culture in a mortar, and injects moderate amounts of the emulsion into the skin of a guinea-pig, one will obtain, as did Mayer and others, principally productive lesions. When, on the other hand, one injects considerable quantities of fresh blood serum culture of great virulence into the peritoneal cavity or circulation of a guinea-pig, actively exudative and necrotic lesions have always been obtained. The acute disease in man seems to follow the latter type.

In the present case many of the lesions were purely exudative and necrotic and any tendency to a proliferative process was entirely obscured by the violence of the reaction.

These features were especially prominent in the muscles, where the initial position of the necrotic process was indicated by the following characters: (1) The dissolution of the muscle fibres, which was often extensive and sometimes complete before any trace of proliferation of fixed tissue cells could be detected. (2) By the presence of hemorrhage from rupture of necrosed capillaries which preceded other changes in the endomysium of muscle fibres. (3) By the comparative absence of local leucocytosis in early lesions. (4) By the sudden comminution of the exuded cells. (5) By the narrow margin which separated necrotic exudate and tissue from practically unaffected tissue. Similar purely necrotic and exudative lesions have been described by Wright in the livers of guinea-pigs.

In most of the lesions an exudative element resembling that of a severe pyogenic process was present in the form of excessive fibrinous deposits in and about the necrotic zone. As noted by several writers, the nodules were often surrounded by a zone of fibrinous exudate, which in the lung sometimes filled a complete circle of alveoli about a small necrotic area. Likewise many polynuclear and mononuclear leucocytes were usually seen in the pulmonary lesions, from which, however, Staphylococcus pyogenes aureus was isolated.

In spite of the undoubted occurrence of processes of the above type there were many features of the lesions even in this very acute case which support Baumgarten's view that there resides in the glanders bacillus a special tendency to induce proliferation of fixed tissue cells and which must throw this micro-organism in the general class with B. tuberculosis.



This relation is strongly suggested by (1) the gross appearance of the glanders nodules, which much resemble miliary tubercles, and has led to the classification of the disease with the infectious granulomata. (2) The absence of general and any marked local leucocytosis must stand as evidence of the same relation, as showing that the characteristic qualities of B. mallei are different from those of all active pus-producing micro-organisms. (3) The microscopical study of the lesions in the lungs of the present case showed absence of any considerable proportion of polynuclear leucocytes and the presence of many large mononuclear cells, which sometimes exhibited the epithelioid form as in miliary tubercles although necrosing too rapidly to permit of satisfactory conclusions on their form or regarding their origin from endothelia or fibroblasts. (4) The presence of large numbers of giant cells recall the giant cells of entirely different appearance, but probably of somewhat similar significance in the miliary tubercle. (5) Although plasma cells were present in the outskirts of most lesions in connective tissue, they were not more numerous than about many purulent lesions excited by the streptococcus or staphylococcus pyogenes.

From these considerations and from the observations that have been so often repeated on the action of less virulent cultures on animals, the writers conclude that the glanders bacillus tends strongly to induce a productive lesion somewhat similar to the miliary tubercle. This tendency appears to be much less pronounced than with the tubercle bacillus, while the necrotic and exudative tendencies of the glanders bacillus are much more prominent than with the tubercle bacillus, and purely necrotic lesions are observed under some conditions. These are practically the same conclusions as those reached by Johne, who finds that there is no single type of lesion which the action of the glanders bacillus invariably follows.

The septicemic character of the present case sets it off at an extreme distance from the usual character of the process excited by the glanders bacillus which has been defined by Baumgarten as a strict tissue parasite. Not a few cases of the same type in man have, however, been described which would seem to show that the human subject is one of the most susceptible of animals to this infection.

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THE FORMATION OF HEMOLYMPH GLANDS FROM ADIPOSE TISSUE IN MAN.[1]

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Recent contributions to our knowledge of pathological conditions of lymphatic structures has led to a more careful examination and study of hemolymph glands. Our knowledge of these structures and their formation may be said to be the result, indirectly, of the early experimental work on the regeneration of splenic tissue. The investigations of Zambecari (1680), Gerlach, Eberhardt, and Phillipeaux upon this subject led to the work of Tizzoni and Filetti (1880), who still thought that the structures they described were newly formed splenic tissue. They noted the red color and increase in size of the retroperitoneal and thoracic glands and attributed these changes to a post-operative lymphadenitis. The fatty marrow of the long bones was transformed into red marrow, and in two of the splenectomized dogs spleen-like nodules were found in the great omentum. They believed this new splenic tissue to be formed directly from the adipose tissue by a process of absorption of the fat, conversion of the fat cells into a reticulum, followed by leucocytic infiltration and active proliferation of the smaller vessels into Malphigian corpuscles. A continued proliferation of the reticulum and cell elements led to the formation of a pulp-like structure containing red blood cells and about which a connective tissue capsule was formed.

Warthin in a recent paper states that Tizzoni's description of the formation of hemolymph glands from adipose tissue is confirmed in every detail by his (Warthin's) experimental work on sheep and goats. (Jour. Medical Research, May, 1902.) All stages of the process were seen, and he observes

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that if the blood sinuses persist the structure of a hemolymph nodule is presented; if the formation of the lymphoid tissue is so great as to reduce the sinuses to capillaries the node assumes the structure of a lymphatic gland.

In the two cases about to be described, the experimental work of the above quoted receives full confirmation in the human subject.

The first and most important of these cases has already been described as a case of adiposis dolorosa with Dr. F. X. Dercum.

Briefly, the clinical history is that of a male, aet. thirty-nine who had suffered for fifteen years from adiposis dolorosa, *i.e.*, a painful swelling of the subcutaneous fat tissue, with a tendency to induration and fatty tumor formation. He also suffered from epilepsy. Death occurred February, 1902, from erysipelas.

The autopsy revealed an adeno-carcinoma of the pituitary body, acute parenchymatous nephritis, and interstitial neuritis of the nerve filaments of the subcutaneous fat.

The subcutaneous fat on the trunk was four inches in thickness. For the greater part it was of normal color and consistence. Several large bright red nodes the size of an orange were present in the incision from the neck to the pubis. These nodules were surrounded by a firm capsule of connective tissue. This capsule, as was also its contents, was rich in nerve filaments and blood vessels. When this nodule was cut across it was found to consist of thousands of small ovoid fat bodies connected with each other by a delicate reticulum of connective tissue, and these joined to form thick gelatinous looking bands which fused with the capsule.

In the immediate neighborhood of these large nodules several small dark red bodies the size of a pea were found free in the fat. The microscopic examination of these smaller bodies revealed the typical structure of hemolymph glands. The gland tissue was surrounded by a capsule of fibrous tissue. At one end of the gland considerable reticular fat tissue was present. The rest of the gland was composed of a dense fine meshed reticulum, the spaces of which were filled with small mononuclear cells. A comparatively large blood sinus filled with erythrocytes encircled the gland immediately beneath the capsule. Smaller sinuses extended in different directions through the reticulum. A striking characteristic of all the sections was the large quantities of granular blood pigment. Only a small portion of this pigment reacted to the test for hemosiderin. At the fat pole of the gland, the prolifieration of the capillary vessels was marked. Small nodes of small round cells were frequent along these new formed capillaries and especially at places where branching had taken place or were about to take place.

The hemolymph tissue was present in large amounts in the large fat nodules. The arrangement of the tissue was somewhat different from that described in the encapsulated gland. The lymphoid tissue here takes the type of an infiltrating character, mainly affecting the capsule of the fat nodule and the larger trabeculæ extending into the fatty ovoid bodies. The structure of the tissue here is the same as that above described, but is much more extensive. The several steps in the formation of the hemolymph tissue from the fat tissue can be carefully studied on account of the loose and open nature of the tissue and the extent of the involvement of the fat nodule.

The first step in the process is probably a chemical change in the fat preliminary to its resorption. Dr. Edsall found a distinct change in the acid values of this fat as compared to the free fat outside of the nodules. This process of resorption of the fat is also assisted by large round vesicular cells, analogous to the compound granule cells of the central nervous system. These cells filled with fat granules formed a striking picture in some of the sections from the second case to be described. The fat becomes lighter in color, the outlines of the cells become irregular in shape, and at the junction of the cells several nuclei are seen in a stellate mass of protoplasm. At the same time the capillaries of the capsule and trabeculæ have extended into this area and large numbers of the proliferating vessels can be seen in a small space. Here and there accumulations of small round nucleated cells are noted around these vessels. As the process advances these accumulations of cells become larger and closer together; the capillary channels widen out; the loose fat reticulum assumes a closer type due to the proliferation of its own cells and the new formed blood channels; the spaces of the reticulum are now filled with the small nucleated type of cell, and the new tissue may be said to be completed as far as a functioning tissue is considered. In all such tissue described as functioning, large quantities of blood pigment in different stages of degeneration can be seen. Like the isolated hemolymph gland, only a small portion of this pigment gives the free iron reaction. While most of the cells followed the type of the lymphocyte, large plasma cells were occasionally seen and a type of cell which gave some of the staining reactions of nucleated red blood corpuscles. Dr. C. Y. White, who carried some of the sections through the differential blood stains, was inclined to regard them as the small mononuclear type of leucocyte, although he was not at all positive in this opinion.

The large vesicular cells filled with fat granules and pigment were more frequent in the infiltrating tissues than in the isolated fully formed hemolymph glands. The cell body was composed of a faint reticulum with an amorphous yellow substance between the fibres of this reticulum.

The second case which presented newly formed hemolymph tissue was also a case of adiposis dolorosa. She was thirty-six years of age, weighed over three hundred pounds, and presented clinically the symptoms of brain tumor. The pathological diagnosis was as follows: Glioma of brain involving the pituitary body, basal nuclei, filling up the fourth ventricle and associated with a dorso-cervical syringomyelia; chronic thyroiditis with calcification and acute localized inflammation of the thyroid gland; cirrhosis of the ovaries; interstitial nephritis in the fat nodules. The changes in the fat tissue were almost identical to those above described. The large granular cells, with indistinct nuclei and filled with pigment granules, were present in large numbers in these tumors. This was especially true of those areas between the fully formed hemolymph tissue and the pale fat tissue. The same transition from normal fat into the hemolymph tissue could be easily followed.

The examination of the spleen in both the above cases gave the same result. The spleen was at least twice the size of the normal organ, the capsule was thickened and a dirty white in color, and was of a firm consistence. The microscopic examination revealed a marked cirrhosis. The trabecular tissue was thickened, and extensive masses of free red blood cells were without the vessel walls. The hemorrhagic extravasation, if it can be so called in a tissue so vascular as that under consideration, was so extensive in some of the sections as to completely overshadow the cellular network.

It is beyond the scope of this paper to consider the relation between the visceral lesions in these two cases, and the transformation of the fat tissue into hemolymph tissue. Whether it is secondary to the splenic lesions, or whether the latter is a part of a general disturbance of the lymphoid tissues dependent on a disturbance of the pituitary body, thyroid, testicle, ovaries, etc., cannot be definitely determined. There is much, however, in the findings here recorded to favor the latter view. Recent work upon the influence of the secretion of the pituitary body, thyroid, and testicle on tissue oxidation and metabolism and the well-known influence of the functional activity of the generative organs upon fat deposition also point to the same conclusion.

THE VITALITY OF BACTERIA FROM THE THROATS OF SCARLET FEVER PATIENTS, WITH SPECIAL STUDY OF STREPTOCOCCI.[1]

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(*From the Memorial Institute for Infectious Diseases, Chicago.*)

Clinical observations appear to indicate that the specific virus of scarlatina is most tenacious of its vitality, withstanding drying in a dark place for a long time. Because of this it was thought that it would be of interest to determine whether any of the bacteria found in the throat in cases of scarlatina are very resistant to desiccation and, if so, which ones they might be. The following investigations were undertaken to determine these points.

The material for study was obtained from the tonsils and pharynx upon sterile cotton swabs, each swab being stored in a sterile, cotton-stoppered test-tube, in a dark place at room temperature. From these swabs cultures were made at intervals until no growth occurred. Loeffler's blood serum mixture was usually employed as nutrient medium, and when no growth was obtained upon it, the swab was immersed in ascites-bouillon in order to detect single organisms which might be overlooked when a solid medium was alone inoculated.

One hundred and forty-five swabs were collected from ninety-five cases of clinical scarlatina, some being very grave, the majority being of medium severity, and many very mild. The material was obtained from the first to the thirty-seventh day of the disease, *i.e.*, from the appearance of the eruption. From eighty-seven of the swabs streptococci were cultivated, while from fifty-eight they were not. This is not, however, to be received as the proportion of cases in which streptococci were present. In several instances swabs were not examined until several days after they had been taken, and although no streptococci were cultivated, they

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may have been primarily present and have died before cultures were made. Antiseptic agents in gargles and sprays may also have influenced the results in some cases. When the growth from a swab contained so few streptococci in comparison with other bacteria that they could not be certainly recognized in the mixed culture, they were considered absent in recording the result. This factor is especially important in instances in which the swab touched the tongue or in which the child gagged when the tongue was depressed. In this connection it is of interest to notice that of forty-one swabs from which cultures were prepared the day on which they were taken forty yielded streptococci, four in pure culture, and most of them in very large numbers compared with other bacteria present, while only one yielded no streptococci. In several instances when one swab yielded no streptococci on cultures, other swabs taken earlier or later from the same case gave them in abundance. In twelve cases from which no streptococci were obtained, a single swab was taken. In seven of these the swabs were sterile when cultures were first made from them, which was from twenty-six to forty-five days after the material had been collected. Making allowance for these sources of probable inaccuracy, it is likely that a very much larger proportion of the cases than the figures indicate really had streptococci in their throats at the time swabs were made.

If we consider the number of swabs from which streptococci were obtained during different periods of the disease, we obtain interesting results. Of one hundred and six swabs taken during the first ten days of the disease, seventy-seven, or thirty-seven per cent, yielded streptococci; of twenty-one taken from the eleventh to the twenty-first day, seven, or thirty-three per cent, yielded streptococci. As the sources of error were the same in all cases, these figures show that the streptococci gradually disappear or become less frequent as the disease passes from the earlier to later stages, and as convalescence progresses.

By making inoculations from the same swab at frequent intervals, it was hoped to learn what bacteria longest withstood desiccation, and also how long streptococci retained their vitality in dried secretions from the throat. In twelve instances swabs which yielded cultures of streptococci, together with other bacteria, later gave a pure culture of streptococci. These twelve swabs were collected during the first eight days of the disease. The time required for swabs which yielded streptococci upon inoculation to become sterile varied from eighteen to ninety days, on an average being about forty-five days. When streptococci were present alone they did not survive longer than when associated with other bacteria. In the examination of cultures made from the swabs at intervals, it was observed that the various diplococci, sarcinæ, and other cocci which were usually present in the early cultures rapidly disappeared. In the cultures which were made from the swabs soon after they were taken, streptococci were usually present in larger numbers than other cocci. After the swabs had been left for a longer time, the cultures showed, beside streptococci, most often bacilli, many of them sporulating, and cocci which grew in large yellow or grayish colonies, such as are commonly seen in cultures made from the air.

In forty-nine swabs which yielded streptococci, together with other bacteria, the streptococci were cultivated as long as any of the associated bacteria, all of them dying about the same time.

These observations show that streptococci are more abundant early in the disease, becoming less numerous as the disease progresses, and that the streptococci resist drying as long as the other bacteria which are usually present in the throat, and in many instances outliving all the other forms. The longest time after which streptococci could be cultivated from the dried material, *i.e.*, ninety days, does not correspond to clinical observations where the scarlatinal virus is said to have produced infection after lying dormant for years. Kurth (Arbeiten aus dem Kais. Gesundheitsamte, 1891, vii, 389) found the streptococcus conglomeratus to remain viable for six weeks or so when dried in air, and he concluded that the transmission of scarlatina corresponds to the vitality of this streptococcus. Holst (Cent. f. Bakt., 1892, xi, 768) demonstrated that streptococci in pus dried in the air remained alive and virulent four and one-half months, but not longer.

Different observers have held various views as to the relationship between streptococci and scarlatina. Whether different forms of streptococci can be recognized by cultural methods, one form of which is peculiar to scarlatina, will be discussed later in connection with a study of streptococci. A few investigators, as Klein and Gordon, believe the specific cause of scarlatina to be a streptococcus. The great majority of those who have written upon the subject believe the streptococcus to be the cause of many of the complications of scarlatina, but they are inclined to give this organism no place in the etiology of the uncomplicated disease. Among those who assign the streptococcus to an important place as an agent of secondary infection are Booker, Crookshank, Fränkel and Freudenberg, Heubner, Hirschfeld, Pearce, Raskin, C. Seitz, Tangl, Wright, and Wurtz and Bourges. Some recent observers, especially Baginsky and Moser, appear to look upon the streptococcus as a most important factor in the causation of scarlet fever. Moser (Wiener klin. Woch., 1902, No. 41, 1053) has no doubt that the scarlatinal streptococcus differs from the others found in human diseases, as erysipelas, phlegmon, etc., and he, as well as Baginsky (Berl. klin. Woch., Dec. 1 and 8, 1902), has undertaken to treat scarlatina with special anti-streptococcic serum.

STUDY OF PURE CULTURES OF STREPTOCOCCI.

Twenty-four pure cultures of streptococci, from as many cases, were studied somewhat in detail. These cultures were obtained from swabs which had been allowed to dry twenty-three to fifty-three days after being taken. The inoculation from the swabs gave growths consisting exclusively or principally of streptococci. By means of a series of dilutions, single colonies were obtained upon blood serum, and by inoculating from these, pure cultures were insured. The cultures agreed in all essential details, only differing in those peculiarities which are variable in the individual culture (coagulation of milk, etc.).

The morphological character varied somewhat with the medium upon which the cocci were cultivated. They usually stained well with the ordinary bacterial staining solutions, carbol-fuchsin and aniline-water solution of gentian-violet staining the cocci intensely and uniformly, while Loeffler's solution of methylene-blue sometimes gave an irregular stain which will be referred to later. The cocci were stained by Gram's method, but the color could be removed if washing in alcohol were sufficiently persisted in.

The growth upon the surface of Loeffler's blood serum mixture consisted of small cocci, quite uniform in size, and occurring singly, in pairs, or in very short chains. The chain formation was very slight. They stained rather poorly but evenly with Loeffler's solution of methylene blue. In the water of condensation the growth was almost exclusively in quite long chains.

Upon plain nutrient-agar, glycerin-agar, and earth-agar, the cocci varied much in size, relatively few being very large. Chains of considerable length were usually observed, and large individuals were often seen as members of a chain which otherwise was composed of small cocci. Sometimes a very large coccus would be located at the end of a chain. The cocci grown upon these media were often stained unevenly by Loeffler's solution of methylene-blue, some individuals in a chain staining well, others not at all or very slightly. The larger forms usually stained better than the smaller ones. Irregularity of staining appeared to be particularly pronounced in cultures made directly from swabs which had been kept for a long time. Sometimes the individual cocci exhibited peculiar forms, being spindle and pear-shaped, and occasionally elongated forms stained in an irregular manner, suggesting the appearance of diphtheria bacilli. Similar irregularities in form, size, and tinctorial properties have been described by Gordon, Baginsky and Sommerfeld, F. Meyer, Stolz, Ohlmacher, and others, and such peculiarities appear to occur in streptococci from various sources, and to be in no way characteristic of streptococci from scarlatina.

When the cocci were grown in fluid media they were arranged in chains which were usually quite long, often reaching across the field of the microscope. Sometimes the chains were well separated, as was usually the case in ascites-bouillon; at other times the chains were interwoven, as in glucose-bouillon. There was no constancy in the length of the chains in the same culture. Aronson, Baginsky, and Meyer, all found great variability in the length of chains in subcultures from the same original culture. Milk cultures yielded cocci of uniform size, arranged in long chains and staining well.

Capsule formation was sometimes noticed, but usually not in any marked degree. In the hanging-drop no active motion was observed.

The growths of these cultures upon various nutrient media corresponded to growths of the streptococcus pyogenes. Slight variations in the reaction of media appeared to exert a marked influence upon the growth, and any considerable deviation from the neutral point on either side interfered with or inhibited it. In speaking of the growths upon various media, unless otherwise stated, the reaction was one per cent acid to phenolphthalein, which is about neutral to litmus.

On the surface of Loeffler's blood-serum the growth was delicate and colorless, and usually consisted of separate, minute colonies. Sometimes the colonies showed some tendency to become confluent, and at times they spread out a little over the surface, instead of remaining the size of a pinhead with slight elevation, as was most frequent. The degree of moisture possessed by the serum seemed to be largely responsible for the variations. In the water of condensation at the bottom of the tube, a profuse, flocculent growth was constantly observed. This was not tenacious and was very readily broken up and distributed uniformly throughout the fluid. A smear preparation from this latter growth showed the flocculent material to consist of long chains of cocci, together with a great deal of granular precipitate. The water of condensation soon became acid to litmus.

Upon the surface of plain nutrient-agar, glycerine-agar, and earth-agar, there was a very delicate, colorless growth. This was often barely perceptible, and varied with different lots of media. Upon glycerine-ascites-agar, the growth was similar to that just described. In glycerine-ascites bouillon the growth was usually abundant. Sometimes there was diffuse cloudiness for forty-eight hours with subsequent collection of the growth at the bottom of the tube, leaving the upper fluid clear. In other instances the upper fluid was clear from the first, the growth appearing only at the bottom. The growth at the bottom of the tube was white, flocculent, or somewhat tenacious. On agitation it rose in the fluid as a mass, but was easily broken up and distributed, usually as a uniform cloudiness and rarely as a finely granular suspension. Coverslip preparations from the precipitate showed a large part of it to consist of granular material together with cocci in long chains. The reaction of the fluid was constantly neutral or very faintly acid to litmus, the acidity never reaching to any considerable degree. Baginsky and Sommerfeld found ascites-bouillon to be alkaline after fourteen days. In sugar-free bouillon, prepared after Theobald Smith's method, growth varied with the reaction and with the presence or absence of sugar. When the reaction was neutral to phenolphthalein and no sugar was added, there was very slight or no growth; if two per cent of glucose was added the growth was fairly abundant. If the reaction was one per cent acid there was a fair growth, but it was much more profuse when two per cent of glucose was added. In the same medium with an acid reaction of two per cent growth was less abundant than in that of one per cent acid, both without and with the addition of glucose. If the reaction was one per cent alkaline, there was very slight or no growth, but if glucose was added considerable growth occurred. When the media contained no sugar the reaction remained almost the same as before inoculation, while in each case when glucose had been added the fluid became acid; or if primarily acid, it became more so. With the production of acid there was less diffuse turbidity and more tendency for the growth to collect as a precipitate. In the alkaline medium with glucose, the growth often assumed the shape of very conglomerative bunches or scales in the form of a precipitate. If a series of tubes of ordinary one per cent acid bouillon containing none and various amounts of glucose were inoculated with the same culture. there was no or very slight growth in the tube without sugar; in the tube containing 0.0625 per cent of glucose, growth was fairly abundant and accompanied by a very feeble production of acid. Here there was more diffuse cloudiness of the medium after twenty-four hours, and much less conglomeration of the growth later than in the tubes containing larger proportions of sugar. It is not unlikely that some of the contradictory statements regarding streptococcus cultures in bouillon are explainable by the varying amounts of muscle-sugar in bouillon prepared from beef. In bouillon to which maltose, saccharose, lactose and levulose, and dextrose were added, the streptococcus grew in a similar manner to that exhibited in glucose-bouillon. From each, acid was produced as in the case of glucose. In bouillon containing mannite, growth was no better than in plain bouillon and no production of acid was observed. Libman (Journal of Medical Research, 1901, vi., 84), in a study of acid production by bacteria in media containing sugars and serum, found that streptococci produce acid from sugars. Page (Jour. of Boston Soc. of Med. Sciences, 1899, iii., 323) found that streptococci cause fermentation of sugars in bouillon, giving rise to from 3.4 to 3.9 per cent of acid. He also concluded that the quantitative determination of the amount of acid produced from each sugar is probably not an important aid in separating varieties of streptococci. The acid production by the streptococci studied by us in glucose bouillon has been quite constantly about one per cent. We have found the character of the growth in bouillon to be inconstant in the same culture.

One which first grew as a very firmly conglomerated precipitate would afterward grow as a flocculent, readily distributed sediment, or as a uniform turbidity. The tendency to produce a conglomerate growth usually became less after prolonged cultivation on artificial media. These observations correspond with those previously made by Aronson, Baginsky and Sommerfeld, Meyer, Vincent, and others. As already stated above, it has been observed that in media containing sugars, with the production of acid there is generally a tendency for the growth to be precipitated, leaving the supernatant fluid clear. Vincent has also noted the occurrence of sedimentation of cultures primarily turbid due to production of acid.

In Marmorek's leucin-glycocoll-bouillon there is first a diffuse turbidity. Later growth slowly collects at the bottom, the upper fluid remaining turbid for a long time. The reaction becomes only faintly acid.

Upon potato no visible growth was observed. In neutral potato-water there was a profuse growth as a precipitate at the bottom, with clear supernatant fluid which soon became strongly acid.

In litmus milk the streptococci grew well. In twenty-four hours at 35° C. the reaction was decidedly acid and the red color became more pronounced for four to six days. There was never any considerable decolorization unless coagulation occurred. Coagulation was usually absent, but occasionally occurred after about a week. After repeated transplantation of cultures the ability to coagulate milk was sometimes acquired or lost.

Growth occurred best at incubator temperature. At room temperature growth was very slow.

The growth in gelatine was limited and no liquefaction was observed.

The vitality of these streptococci upon artificial nutrient media varied with the medium, and the same culture survived longer upon a given medium at one time than at another. In glycerine-ascites bouillon, the streptococci were usually alive at the end of thirty days, but dead in sixty days.

On Loeffler's blood serum mixture they died much more quickly, being unable to grow after five to thirty days. In potato-water they died in a few days. Cultures in two per cent glucose bouillon were dead in two or three days, but if carbonate of calcium was added to the media before it was sterilized, the cocci remained alive for more than seventeen days. Reference has already been made to the vigor with which these streptococci convert sugar into acid. It is also true that the streptococci die more quickly in media containing sugar which is converted into acid. When carbonate of calcium was added to the glucose bouillon, the acid was largely neutralized as produced, and the reaction of the medium never becoming acid to any considerable degree, the bacteria survived longer.

Twenty-one cultures were tested as to their vitality in milk. Of these, two were dead in sixty days and four in eighty days. The remaining ones were dead after one hundred and forty to one hundred and sixty days. One culture is still alive after two hundred days. It was not found that life was lost sooner when the milk was coagulated. A second series of tests were made with twenty-two cultures, using in part the same, together with additional cultures, after they had been grown on artificial media for a longer time, having passed through ten to fifteen generations on various media. Of these, eleven were dead in eighteen days, six in fifty-four days, two in seventy-six days, and the remaining three were alive after ninety days. The resistance of the bacteria would thus seem to have been reduced by prolonged cultivation.

Two cultures which had been grown in ascites-bouillon for about six months were found to produce a brown pigment when inoculated upon Loeffler's blood serum. Neither of them possessed this property originally, and one lost it on being transferred to fresh blood serum, resuming its original colorless growth. The other continued to produce brown pigment when transferred to fresh medium. The production of brownish or reddish pigment by streptococci has been previously observed.

As the result of our studies it has been impossible to differentiate the streptococci from scarlatinal anginas from streptococci from other sources. All the cultural and morphologic properties are duplicated by the ordinary streptococcus pyogenes. A part of the cultures have been tested as to their effects upon animals, and have been found to possess slight virulence for rabbits and guinea-pigs. Meyer has found agglutination tests of value in the differentiation of certain pathogenic forms of streptococci, but more study is required before anything final can be decided regarding such phenomena. Baginsky found scarlatinal blood serum without agglutinating action upon streptococci. Moser, however, says that his serum agglutinated streptococci from scarlatinal cases in a very different manner from other streptococci. As few details are given, it has been impossible to verify or disprove these assertions.

Conclusions: —

1. Streptococci are almost always if not constantly present in the throat in cases of scarlatina. In the early stages they are usually in very large numbers, becoming less numerous as the disease progresses.
2. The streptococci in the throat of scarlatina patients resist drying as long as the other bacteria usually present, and they often outlive all other forms, being alive as long as ninety days after the material is collected.
3. These streptocccci remain alive for a long time in milk.
4. A small amount of sugar in nutrient media increases their value for the cultivation of streptococci.
5. Streptococci from scarlatinal anginas are not different from streptococci from other sources so far as cultural and morphologic peculiarities are concerned.

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UPON THE PLURALITY OF CYTOLYSINS IN NORMAL BLOOD SERA.[1]

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Our studies upon the hemolytic cytolysins contained in snake sera[2] and some recent experiments, to be given in a separate paper, upon the cytolysins of snake venom, led us to study the action of several kinds of blood sera upon such visceral cells as spermatozoa, testicle, liver, and kidney cells. The study quickly proved the natural occurrence of a number of cytolysins in the sera which are either wholly or partially distinct from the hemolysins which these sera also contain. In this study we employed sera from the dog, ox, rattlesnake (*Crotalus adamanteus*), and mudpuppy (*Necturus maculatus*).

Methods of study. — The kidney and liver to be examined are passed through a fine sausage machine and the pulp is shaken vigorously in a large test tube with sterile saline (0.85%) solution. On standing, the emulsion separates into three layers: a lower layer in which the large fragments of tissue, a medium in which the separated cells, and an upper in which fragments of cells and blood corpuscles are contained. The upper and lower layers are discarded and the middle one employed. A second vigorous shaking of the lowest portion with a fresh quantity of saline solution will yield another quantity of suspended cells. The emulsion to be employed is passed through sterile gauze to remove any large fragments of tissue; and a concentration is chosen for experiment in which the fluid is opaque and milky from the suspended cells.

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[2] Flexner and Noguchi. Snake Venom in Relation to Hemolysis, Bacteriolysis, and Toxicity, Univ. Penn. Med. Bulletin, 1902, xiv, 438; The Constitution of Snake Venom and Snake Sera, Univ. Penn. Med. Bulletin, 1902, xv, 345.

The testicle cells, including spermatozoa, are obtained by shaking the testes in saline solution. In the case of the dog and rabbit, on account of their fibrous character the organs are first sliced; in that of the rat and guinea-pig, the separation by shaking can be accomplished after merely opening the tunica albuginea.

The serum is used in undiluted condition; the amount of emulsion added is approximately five per cent of the volume of serum. The mixtures are kept first at room temperature, after which they are placed in an ice-chamber for variable lengths of time when longer periods of contact are employed.

TABLE I. — DOG'S SERUM.

Source of Cells and Duration of Action of Serum.	ORGANS.			5 per cent Blood.
	Liver.	Kidney.	Testis.	
Rat.	Control: Large polygonal and granular cells; nuclei distinct. After 6 hours little change.	Control: Cells somewhat variable in size with granular protoplasm; after 6 hours no disintegration.	Control: Large round cells with resting and dividing nuclei; contours distinct; few granules in protoplasm. Many spermatozoa. After 6 hours little change.	
Time. 30 minutes.	Cells are much cleared and a slight deposit has formed.	Marked agglutination; cells rendered clearer.	Marked agglutination.	Hemolysis in 7 minutes.
3 hours.	No preserved cells, but only fragments of cells; few granules and fat globules.	Cells in masses have lost largely their outlines; free nuclei and many free granules are present.	The testicular cells are largely disintegrated; free nuclei and granules occur; spermatozoa coiled and broken.	

TABLE I. — *Continued.*

Source of Cells and Duration of Action of Serum.	ORGANS.			5 per cent Blood.
	Liver.	Kidney.	Testis.	
Guinea-pig.	Control: Cells free and in masses; protoplasm finely granular with small fat globules. Some free nuclei.	Controls: Cells granular; many free nuclei and some free granules.	Control: Cells free and some in masses; protoplasm clear; outlines distinct. Many spermatozoa present.	
Time. 30 minutes.	Cells swollen; no disintegration noticeable.	Little change.	Little change.	Hemolysis in 3 minutes.
1 hour.	Most free cells have disappeared; large cell-masses indistinct; nuclei invisible.	Marked agglutination; swelling of cells and nuclei; partial disappearance of granules.	Testicular cells swollen; nuclei indistinct.	
3 hours.	Almost complete disappearance. Few indistinct cells remain.	Marked disintegration of cells.	Cells broken up and spermatozoa reduced in number.	
Rabbit.	Control: Free and combined granular polygonal cells, and some free nuclei and granules.	Control: Some free and many combined granular cells; free granules and nuclei.	Control: Same as guinea-pig's emulsion.	
Time. 30 minutes.	Marked agglutination; no disintegration.	Little change.	Slight agglutination; no solution noticeable.	Hemolysis in 4 minutes.
3 hours.	Large disappearance of cells; remaining cells swollen and indistinct.	Little solution, but great swelling with obliteration of outlines of cells.	Many of the cells have disappeared; remaining nuclei swollen; spermatozoa little altered.	

TABLE I. — *Continued.*

Source of Cells and Duration of Action of Serum.	ORGANS.			5 per cent Blood.
	Liver.	Kidney.	Testis.	
Rattlesnake. *Time.* 3 hours.	Moderate agglutination; swelling of cells but doubtful solution.	Little alteration.		Trace of hemolysis.
Necturus. *Time.* 3 hours.	Marked agglutination; little or no alteration.			No hemolysis.
Dog. *Time.* 4 hours.	No effect.	No effect.	No effect.	No hemolysis.

TABLE II. — OX'S SERUM.

Source of Cells and Duration of Action of Serum.	ORGANS.			5 per cent Blood.
	Liver.	Kidney.	Testis.	
Rat. *Time.* 1 hour.	Marked agglutination; swelling of protoplasm with obscuration of nuclei; nuclei swollen.	Marked agglutination; no solution.	Testicular cells swollen and partly disintegrated; many free nuclei; spermatozoa unchanged.	Marked agglutination; hemolysis in 2 hours and 20 minutes.
4 hours.	Slight disintegration; otherwise unchanged.	No further change.	Moderate agglutination; disintegration advanced in testicular cells.	

TABLE II. — *Continued.*

Source of Cells and Duration of Action of Serum.	Organs.			5 per cent Blood.
	Liver.	Kidney.	Testis.	
Guinea-pig.				
Time. 1 hour.	Moderate agglutination; moderate swelling of cells; no solution.	Agglutination; swelling of cells; nuclei less distinct; no solution.	Agglutination; swelling of cells, but no solution.	Marked agglutination; hemolysis in 4 minutes.
4 hours.	Swelling increased; granules appear coarser; no solution.	Swelling increased and some fragmentation.	Swelling increased; no solution.	
Rabbit.				
Time. 1 hour.	Swelling of cells, but no solution.	Agglutination and swelling, but no solution of cells.		Hemolysis in 5 minutes.
4 hours.	Agglutination, but no further change.	Idem.		

TABLE III. — RATTLESNAKE'S SERUM.

Source of Cells and Duration of Action of Serum.	Organs.			5 per cent Blood.
	Liver.	Kidney.	Testis.	
Rat.				
Time. 30 minutes.	Moderate agglutination; advanced solution.	Marked agglutination; cell-masses swollen and clear; beginning solution.	Marked agglutination.	Hemolysis in 7 minutes.
3 hours.	No intact cells remain, but a few broken cells, granules, and fat globules can be seen.	Increased swelling and advanced solution. Some free nuclei and granules remain.	Extensive disappearance of cells; some free nuclei and granules remain; spermatozoa much broken.	

TABLE III.— *Continued.*

Source of Cells and Duration of Action of Serum.	ORGANS.			5 per cent Blood.
	Liver.	Kidney.	Testis.	
Guinea-pig.				
Time.				
30 minutes.	Moderate agglutination.	Slight agglutination; some swelling of cells.	Moderate agglutination.	
3 hours.	Cells swollen and show slight evidences of disintegration.	No intact cells present, but only débris remains.	A few fragments of cells only remain.	Hemolysis in 3 minutes.
Rabbit.				
Time.				
30 minutes.	Marked agglutination and much swelling of cells.	Much swelling and clearing.	Marked agglutination.	
3 hours.	Almost complete solution; only free nuclei remain.	Nuclei and occasional cell and granules only visible.	All cells have disappeared after 3 hours, but occasional nucleus remains.	Hemolysis in 3 minutes.
Dog.				
Time.				
30 minutes.	Moderate agglutination.	Moderate agglutination.		
2 hours.	Much disintegration; free nuclei and granules.	Much solution; free nuclei numerous.	Spermatozoa broken up; deposit of nuclei and cell granules.	Hemolysis in 5 minutes.
6 hours.	Almost complete disappearance. After diluting the serum one-half with saline solution, the solution is delayed and rendered imperfect.	Only nuclei and débris remain. Dilution of one-half delays the action.		
Necturus.				
Time.				
30 minutes.	Slight agglutination.			
3 hours.	Much swelling; doubtful solution.			Hemolysis imperfect.

TABLE IV. — NECTURUS' SERUM.

Source of Cells and Duration of Action of Serum.	Organs.		5 per cent Blood.
	Liver.	Testis.	
Rat. *Time.*			
30 minutes.	Much cleared.	Swollen and cleared.	
3 hours.	Partial solution; undissolved cells, nuclei, and granules remain.	Largest cells disappeared; many swollen and indistinct cells remain. Spermatozoa broken.	Hemolysis in 8 minutes.
Guinea-pig. *Time.*			
30 minutes.	Moderate agglutination.	Swollen and cleared.	
3 hours.	Cells much broken; some indistinct but unbroken cells remain.	Only a few undissolved cells remain; some débris.	Hemolysis in 4 minutes.
Rabbit. *Time.*			
30 minutes.	Marked agglutination.	Markedly cleared.	
3 hours.	Much swelling, but doubtful solution.	Advanced disintegration and solution.	Hemolysis in 3 minutes.
Dog. *Time.*			
30 minutes.	Marked agglutination.	Slight agglutination.	
3 hours.	Much swelling, but doubtful solution.	Much disintegration; a few swollen and indistinct cells and many free nuclei remain.	Hemolysis in 6 minutes.
Crotalus. *Time.*			
3 hours.	No pronounced change.		Hemolysis imperfect.

From the experiments given the conclusion that normal blood sera of warm and cold blooded animals contain agglutinines and solvents for visceral as well as blood cells can be drawn. The amounts and activities of the solvents vary not only with the origin of the sera but also with the species of animals from which the cells have been obtained.

Having established this fundamental fact, the next inquiry was to ascertain whether the agglutinines and solvents are identical for all the cells tested or whether they are absolutely or relatively distinct. The study of this question was conducted by absorbing the agglutinines and solvents for a given kind of cell by exposing a mixture of the emulsion of cells and the serum for one hour at the temperature of the ice chamber after which the fluid was separated by centrifugalization. It was found that this short immersion at 0° C. was sufficient to remove the solvents for given cells without removing the dissolving power of the serum for all the cells.

These experiments were carried out with dogs' and rattlesnakes' serum and rabbits' and dogs' cells as given in Tables V. and VI.

TABLE V. — ANALYSIS OF DOGS' SERUM WITH RABBITS' CELLS.

Manner of Absorption.	Liver.	Kidney.	Testis.	Blood.
Treated with washed blood corpuscles.	Marked disintegration after 6 hours.	Moderate disintegration after 6 hours.	Moderate disintegration after 6 hours.	Marked agglutination; no solution.
Treated with liver cells.	No change.	Moderate disintegration in 6 hours.	Moderate disintegration in 6 hours.	Complete solution in 4 minutes.
Treated with testicle cells.	Moderate disintegration in 6 hours.	Moderate disintegration in 6 hours.	No change.	Complete solution in 5 minutes.
Treated with kidney cells.	Moderate disintegration in 6 hours.	No change.	Moderate disintegration in 6 hours.	Complete solution in 4 minutes.

TABLE VI.—ANALYSIS OF RATTLESNAKES' SERUM WITH DOGS' CELLS.

Manner of Absorption.	Liver.	Kidney.	Testis.	Blood.
Treated with washed blood corpuscles.	Much disintegration and solution after 6 hours.	Much disintegration and solution after 6 hours.		No change.
Treated with liver cells.	No change.	Disintegration and solution after 6 hours.		Complete hemolysis in 7 minutes.
Treated with kidney cells.	Disintegration and solution after 6 hours.	No change.		Complete hemolysis in 6 minutes.

The next step was to remove successively the solvents for the several kinds of cells from the serum, and to test the supernatant of each fraction with all the kinds of cells. The method employed was as follows: four cubic centimeters of dogs' serum was treated with one-half of a cubic centimeter of emulsion of liver, kidney, testis, and blood in succession, separately, and in combination, the whole volume being made up with saline solution to eight cubic centimeters in each case. The supernatant fluid obtained by centrifugalization represented half-strength serum, and its solvent power was compared with controls of normal serum diluted one-half with saline solution.

TABLE VII.—ANALYSIS OF DOGS' SERUM WITH SUCCESSIVE FRACTIONS OF RABBITS' CELLS.

	Liver.	**Kidney.**	**Testis.**	**Blood.**
Time. *Normal Serum Control.* 4 hours.	Cells swollen, outlines pale and indistinct; granules in part dissolved.	Granules in cells largely dissolved; cells rendered clear and indistinct; nuclei visible.	Much disintegration of testicular cells; free nuclei and granules present; partial disappearance of spermatozoa.	Hemolysis in 6 minutes.
1st fraction. Minus liver cells; 4 hrs.	No change.	Agglutination; much swelling; partial disappearance of protoplasm, but nuclei and some granules remain.	Disintegration less advanced than in control.	Hemolysis in 6 minutes.
2d fraction. Minus liver and kidney cells; 4 hrs.	No change.	Partial disappearance, but many intact cells remain.	Much disintegration, but some nuclei and granules remain.	Hemolysis in 7 minutes.
3d fraction. Minus liver, kidney, and testis cells, 4 hours.	No change.	Partial disintegration.	Partial, but much reduced disintegration.	Hemolysis in 7 minutes.
4th fraction. Minus liver, kidney, testis, and blood cells; 4 hours.	No change.	Partial disintegration.	Partial disintegration.	Hemolysis in 30 minutes.

This experiment shows partial absorption of the several solvents except the one for liver cells which was completely removed. A second experiment with twice the quantity of the emulsions was made in the hope that the entire solvent-content could be extracted. That this is possible the next table shows.

TABLE VIII.—ANALYSIS OF DOGS' SERUM WITH SUCCESSIVE FRACTIONS OF RABBITS' CELLS.

	Liver.	Kidney.	Testis.	Blood.
Time. *Normal Serum Control.* 3 hours.	Much swelling and disintegration of cells.	Much swelling of protoplasm and solution of granules. Nuclei clear and distinct.	Great disappearance of cells and granules. Some indistinct cells remain.	Hemolysis in 5 minutes.
1st fraction. Minus liver cells; 3 hrs.	Slight swelling, but very little change.	Like control.	Like control.	Hemolysis in 6 minutes.
2d fraction. Minus liver and kidney cells; 3 hrs.	Like previous one.	No disintegration.	Like control.	Hemolysis in 6 minutes.
3d fraction. Minus liver, kidney, and testis cells, 3 hours.	Little or no change.	No disintegration.	Some swelling, but no disintegration of cells.	Hemolysis in 10 minutes.
4th fraction. Minus liver, kidney, testis, and blood cells, 3 hours.	No change.	No change.	Like previous one.	Hemolysis, trace.

From this experiment the conclusion is justified that the solvents in dogs' serum for several kinds of rabbits' cells are perhaps not absolutely specific, but yet to a large extent special for the different cells. The next question to be considered is the nature of the mechanism through which this solution is brought about and its relation to the mechanism of hemolysis.

For the purposes of this inquiry the solvent effect of rattlesnakes' serum upon dogs' cells was studied. The serum heated to 56° C. for thirty minutes loses its solvent action, and this action could, it was found, be restored by the addition of so-called pure complement obtained by treating the fresh serum at 0° C. with emulsions of cells. The deduction that the solution of cells of organs is brought about by the interaction of an amboceptor and a complement could therefore be made. Table IX. gives the results obtained in these experiments.

TABLE IX. — ACTION OF HEATED (56° C.) RATTLESNAKES' SERUM ON DOGS' CELLS AND THE REACTIVATION WITH CROTALUS COMPLEMENTS.

Solution to which Exposed.	Liver.	Kidney.	Blood.
Heated serum.	Agglutination; no other change.	No change.	Marked agglutination; no hemolysis.
Pure complement.	No change.	No change.	No change.
Fresh serum.	Marked disintegration.	Marked disintegration.	Hemolysis in 7 minutes.
Heated serum and pure complement, equal parts.	Marked disintegration.	Marked disintegration.	Hemolysis in 10 minutes.

The time duration of the tests with the cells was six hours.

While the heating of the serum and destruction of the complement suffice to prevent, in spite of the presence of a small amount of native serum in the organic emulsions, all solution of the cells, the heating of the cellular emulsions to 58° C. for thirty minutes does not prevent the solvent action of rattlesnakes' serum upon dogs' cells.

SUMMARY.

The studies given were undertaken with the view of determining the existence in normal blood-sera of cytolysins for other cells than red and white corpuscles. Sera from warm and cold blooded invertebrates — rattlesnake and dog — as well as from *necturus*, were chosen for the experiments, and the results demonstrated the occurrence in them of solvents for a wide variety of animal cells.

This fact having been established, the next undertaking was the determination of the specificity of the solvents for the different cells. The removal of a part of the solvents by means of certain cells does not prevent action of the treated sera upon still other cells, from which it could be concluded that the solvents are at least specially adapted to given cells; while a general reduction in activity of the sera which have lost a part of their solvents would also suggest that these are not specific in the strict sense.

The mechanism of the solvent action is probably identical with that of serum-hemolysis and depends upon the interaction of amboceptors and complements. That the amboceptors are multiple in each serum would seem to be proven by the absorption tests; but whether the complements are also multiple has not been considered. It is highly probable that they are. Moreover, the inability of the serum amboceptors to unite with alien complements in the organic emulsions would indicate that they are isocomplementophilic.

In considering the reliability of the experiments here recorded, note should be made of the fact that observations upon the organic emulsions were made in test-tube reactions as well as under the microscope. But on account of the difficulty of determining with the naked eye the degree of solution and other changes taking place in the emulsions, recourse must be taken to the microscopical examination. The results of the two series of observations were, however, in accord.

This paper is intended as a contribution to the study of the plurality of the natural cytolysins in blood sera and as a forerunner of our studies, along the same line, with snake venom.

THE AGGLUTINATION AFFINITIES OF RELATED BACTERIA PARASITIC IN DIFFERENT HOSTS.*

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The existence of races of bacteria indistinguishable among themselves, biologically, except in inconstant cultural details, and which attack different species of animals, is one of the facts gained by a comparative study of infectious diseases. Among the more important of those species of bacteria of which a number of races have now been distinguished are the tubercle bacillus, the bacillus of rabbit septicemia, the hog cholera or enteriditis bacillus, and the colon bacillus. There may, perhaps, be some question as to the correctness of the use of the word "species" and variety or race among these bacteria. Thus in the colon group, transition forms are from time to time isolated, which almost bridge the gap between the true colon bacillus and the enteriditis or hog-cholera group of bacteria. Here it is manifestly impossible to draw a sharp line between the one and the other group, especially when everything points to the inference that parasitic forms are being gradually evolved from the saprophytic forms.

The discovery of these so-called races in different species naturally raises the question as to their source. Are they parasitic forms which have descended from saprophytic forms in the same host-species, or have they been transmitted from

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* NOTE. The observations recorded in the following pages were begun in 1898, and continued in a more or less desultory manner until 1901, when the assistance of the Rockefeller Institute enabled me to associate Dr. Reagh in the work and bring it to a preliminary conclusion. During the year 1899 I received much help in carrying out the preliminary tests from J. Reverdy Stewart, then assistant in the antitoxin laboratory. — T.S.

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other species accidentally, and, perhaps, modified thereby? In other words, is their resemblance due to similarity of conditions obtaining in different species, or are they parasites of a few species which have become accidentally diffused over many species and modified by them? This is a fundamental question and it is the essence of the important subject of the intercommunicability of human and animal tuberculosis.

Of the means of differentiating such races from one another, inoculation of susceptible animals, in addition to the study of morphological and biological characters, has been the most reliable guide. This, however, has been felt to be unsatisfactory in itself for various reasons. To it must now be added the more delicate agglutination test which bids fair to prove superior in its accuracy to the inoculation tests and which cannot be omitted from any studies of bacteria laying claim to thoroughness.

In the course of a number of years the accumulation of cultures of the hog-cholera group having close biological affinities made it seem desirable to subject them to a comparative agglutination test. Cultures were on hand from man, the pig, the guinea-pig, and the spermophile. The main question which presented itself was to find out what agglutinative relationships, if any, existed between these cultures derived from four different hosts. If it should be found that the host in the course of time places its stamp upon the agglutinative properties of the bacteria parasitic in it, it might be possible to trace the source or original host of any given bacillus which had accidentally invaded another species, as in the cases of meat-poisoning, psittacosis, and paracolon infections in man. We are aware of the existence of similar investigations by others devoted to the same group of bacteria so peculiarly adapted to such studies owing to their sensitiveness to the agglutinative reaction. The following studies are, however, made in the main from the standpoint of the host and, in part, with cultures as yet undescribed.

I. BRIEF HISTORY OF THE CULTURES INCLUDED IN THE COMPARATIVE TESTS.

A. *Motile bacilli isolated from animals. Produce gases and acids in dextrose bouillon, but not in saccharose or lactose bouillon. Gas formula, roughly,* $\frac{H}{CO_2} = \frac{2}{1}$. *Total acidity produced in one per cent dextrose, is four to six per cent of a normal solution.*

I. Hog cholera *a* bacilli corresponding to the type *a* as first described by one of us[1] in 1885 and more recently with other varieties in 1893.[2] This type is of maximum virulence to rabbits. When freshly isolated, 1 cc. of a $\frac{1}{100000}$ dilution of a twenty-four-hour bouillon culture is fatal to adult rabbits within seven to nine days. For guinea-pigs the fatal dose is much higher, probably between 0.1 cc. and 0.3 cc. Of this group were tested the following cultures:

Hog cholera, Maryland (h. c , Md.); isolated in 1898 by Dr. C. F. Dawson.
" " Arkansas; isolated by Dr. Dinwiddie in 1899.
" " Minnesota; 1894.
" " Massachusetts; isolated by one of us from an outbreak in Danvers, 1898.
" " Nebraska; isolated by one of us[2, 3] in 1886.

II. Swine dysentery bacilli. Closely related to hog cholera *α*, but of minimum virulence to rabbits and guinea-pigs. Grow more vigorously than Group I. on gelatine and in bouillon. Colonies simulate those of B. coli. These are probably transition forms standing between B. coli and the highly parasitic type of Group I. Similar forms were described by one of us[2] as *β*, *γ*, etc. Though they might be included as weakly virulent forms under Group I., yet their association with a true dysentery in swine makes it desirable to keep them separate for the present. Of this group two cultures were used:

Swine dysentery *a*; isolated by one of us from an outbreak in Brookline, Mass., 1899.
" " *β*; isolated from an outbreak in Boston, Mass., 1901.

These will be described more in detail in another publication.

III. Bacillus of guinea-pig disease.[4] A bacillus isolated from a guinea-pig in 1896, and then called pseudo-tuberculosis, owing to the presence of large tubercle-like foci in the liver and spleen which were necrotic-suppurative in character. This is evidently the same as the bacillus isolated by Dr. Edward P. Carter at Johns Hopkins University in 1897, a culture of which was kindly sent to me by Dr. Carter, and another recently by Dr. Harris, for comparison with our bacillus. As Dr. Carter did not publish his studies upon this organism, we may state here that in a personal communication Dr. Carter called it the bacillus of infectious endometritis in guinea-pigs. It was isolated from a spontaneous epizoötic in which the females chiefly were carried off by it. They always showed an acute endometritis. A study of our bacillus and of Dr. Carter's bacillus showed no appreciable differences. They are designated in the following pages as "guinea-pig disease *α* and *β*."

IV. Spermophile bacillus isolated in 1897 by Dr. Nelson and Professor Piper, of the Washington Agricultural College, from *Spermophilus townsendi*, during an epizoötic among these animals.* This bacillus is less pathogenic to rabbits than Group I., but somewhat more so to guinea-pigs. Growth in culture media resembles in vigor that of swine dysentery bacilli.

Cultures of *B. enteriditis*, *B. typhi murium*, and the many other forms isolated from cases of meat-poisoning, and evidently of animal origin, were not included in these observations.

B. *Motile bacilli of the same character as A, but isolated from man.*

V. *Bacillus icteroides* obtained through the kindness of Dr. Walter Reed. Of this group two cultures were included: *B. icteroides*, original (Sanarelli), and *B. icteroides*, Santiago (Agramonte, 1898). These cultures grew feebly in gelatine, resembling in this respect Group I. There were certain minor differences between the two stocks, such as the formation of minute granules, probably crystals, in the immediate environment of the Sanarelli culture and not noticed in the Santiago culture.

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* Personal communication.

VI. Paracolon bacilli isolated from the gall bladder and the contained gall stone by Dr. M. W. Richardson in a case of cholecystitis in 1899. The bacillus from the stone developed a surface membrane in bouillon very promptly; the other did not. The virulence toward guinea-pigs of these two cultures was such that 0.1 cc. of a twenty-four-hour bouillon culture was fatal within two days when injected into the abdomen. The subcutaneous injection produced marked fever and loss in weight. In rabbits the subcutaneous inoculation showed that these bacilli were less virulent than the hog-cholera bacillus *a*.

C. *Several varieties of B. coli.*

VII. These included bacilli ranging from the most saprophytic, which ferment three sugars, to those which ferment only dextrose in the typical manner. These were isolated by one of us (T. S.) from pigs in the spring of 1902, and they will be described more fully farther on. The culture chiefly used in the comparative tests with the other groups is a typical colon bacillus isolated from a pig in 1900. It acts upon saccharose.

D. *Several cultures of the typhoid bacillus.*

VIII. Of this group only the true typhoid bacillus was included. Five different stocks from the Massachusetts General Hospital and the Boston City Hospital were used.

In addition to these, several other cultures were from time to time drawn into the investigation, such as a non-motile variety of the hog-cholera bacillus,[5] the bacillus of dysentery (Shiga) obtained from Dr. Flexner, and a non-motile typhoid-like bacillus of fowls.

II. METHODS EMPLOYED.

A variety of methods and procedures have been published by different investigators which have had their origin largely in the conditions of the problem to be investigated and in the amount of blood serum at the disposal of the investigator. When only small quantities of serum are available, the method is necessarily different from that utilizing larger amounts.

In our own work, the microscopic method was at first used extensively. Blood was drawn from the ear of rabbits and guinea-pigs, and the serum used to prepare dilutions in normal salt solution from $\frac{1}{5}$ to $\frac{1}{4000}$ and above. Each culture was tested as follows: a loop of serum and a loop of the culture were placed on a cover-glass and intimately mixed. The cover-glass was then inverted over a concave slide having a little vaseline smeared around the edge of the concavity. This hanging drop was then examined with an oil immersion lens from time to time and the changes noted. The examination was continued until a dilution was found which indicated the limit of clumping, *i.e.*, in which the changes indicated that further dilutions would have no effect. The final dilution was always one-half of the serum dilution used. Thus, if a loopful of a serum dilution of one-fifth was mixed with the same quantity of a given culture, the resulting dilution was one-tenth.

Though the examination with high powers seems the most accurate method, yet it has certain disadvantages and it was replaced by the following macroscopic method, requiring at least four cubic centimeters of blood serum for a test: Guinea-pigs and rabbits were immunized, and after a certain time they were chloroformed, the chest wall removed in part, the heart incised, and the blood flowing into the pleural cavity quickly drawn up with a pipette provided with a rubber bulb.

To make the test, bouillon cultures eighteen to twenty-four hours old were employed. Small test tubes about 1 cm. in diameter were ranged in rows in holes in a tin tray or wooden block, each properly labelled. Dilutions of serum in normal salt solution were prepared in glass-stoppered graduates from $\frac{1}{5}$ to $\frac{1}{8000}$ or more, according to the degree of immunization attained. Into each test tube was put 1 cc. of some serum dilution and 1 cc of the culture fluid. Thus, adding 1 cc. of $\frac{1}{5}$, $\frac{1}{10}$, $\frac{1}{25}$, $\frac{1}{50}$ dilution to 1 cc. of culture fluid, yields dilutions of $\frac{1}{10}$, $\frac{1}{20}$, $\frac{1}{50}$, and $\frac{1}{100}$, respectively. After the dilutions were prepared, the block holding the tubes was placed in the incubator at 36^{c}–37° C. for about two hours. An examination of each tube was then made with the naked eye and with a hand lens and the results noted. The examination was repeated after about one to two hours. If the clumping in the highest member of any series was still pronounced, further dilutions were prepared, until either the final tube failed to show any changes, or else the clumping indicated that the limit had been attained.

While the microscopic method enables us to recognize small and beginning clumps, yet it fails to give us that survey which the test tube and the hand lens afford. A few large clumps among many free bacilli may escape attention in the hanging drop, but not in the small test tube. Again, the occasional occurrence of bouillon cultures in which the bacteria appear in a finely clumped or powdery state is better recognized and estimated in the test tube. By always having control tubes for each culture, errors are thus promptly eliminated. Excessively fine powdery precipitates are easily recognized in strong transmitted daylight with a good hand lens.

It should be said at the outset that for preliminary trials the microscopic method would be the most desirable, but for final tests, aiming at the agglutinative limit for each culture, the test tube method is the most satisfactory, though it may fail to display the smallest clumps. It is, however, a more expensive method, requiring much glassware. In the tests recorded in the table, over sixty pieces of glassware (including the small test tubes, culture tubes, graduates, and pipettes) were used in each test.

It was soon found that there were distinct agglutinative relationships between the races having the fermentative characters of the hog-cholera group, and that it would be necessary to make accurate quantitative determinations. Hence the method of limits was used throughout, it being assumed that this would form the sharpest and clearest means of estimating the agglutinability of the various races and species.

It is well known that different amounts of agglutinins produce quite different appearances in cultures. Thus a relatively large amount of serum will soon completely clump and cause to subside all the bacteria in a given mixture, so that we find, after one or more hours, a white, snowy deposit in the bottom of the test tube and a clear fluid above. With diminishing amounts of serum, the subsidence becomes less and less complete. The suspended bacteria are in clumps of varying sizes, and the suspending fluid may be clear, or where serum in still higher dilutions is employed, the fluid may be cloudy.

The element of time is also of value in that the process of clumping appears more and more tardily with the lessening amount of serum. After three hours at 37° C. the clumping process becomes masked by the multiplication of bacteria in the higher dilutions. The limit chosen by us was the appearance at the end of three hours of a very fine, powdery or granular precipitate distinctly seen with a hand lens, but not detected in higher dilutions or control tubes. This stage may be regarded as indicating the point where the agglutinins have all been taken up.

In some cultures the limit was not as clearly defined as in others. Thus it has happened that in mixtures of certain seta and certain cultures, large flocculi would form while the fluid remained clouded. The bulk of the bacteria remained free and active as shown by the microscopic method. In such cases, the limit was taken to be at that point beyond which flocculi no longer appeared.

The partial clumping in certain cases persisted through a series of successive dilutions, thus making the limit a rather broad, hazy band instead of a sharp line. It aroused the suspicion that possibly, in spite of all care, the cultures used might be mixed or impure. Gelatine roll cultures were, thereupon, made of all suspected cultures and the colonies carefully scrutinized. From such rolls, sub-cultures from one or more colonies, manifestly like all the rest, were made and these sub-cultures tested with the original cultures in parallel series. In the course of this special investigation, it was found that while in nearly all cases the sub-cultures agreed absolutely with the parent culture in agglutinative characters, yet in a few instances the sub-cultures in bouillon were slightly different from the original in appearance, and the agglutinative limit different. But in these few cases the evidence pointed not to mixed cultures but to modifications going on in the cultures in virtue of which several possibly degenerated varieties had appeared. The most striking instance was that of *B. icteroides* (Sanarelli). The gelatine roll cultures were characterized and distinguished from those of all other cultures by the granular zone formed about the colony, due probably to precipitates in the gelatine. Yet in bouillon sub-cultures of some of these, the growth was wholly in clumps in a nearly clear fluid. The difficulty in obtaining a uniform clumping is thus, in part at least, due to changes going on in artificial cultures of long standing whereby the motility becomes impaired and, perhaps, the cell wall more adhesive. This, however, does not explain the condition in all cases, and the partial clumping must be explained in other ways. In the case of one of the hog-cholera cultures, it was hoped to improve the reaction by passing it through a rabbit, but both the original and the rabbit culture acted precisely alike. This problem will be presented again in a later paper. It has many things in common with the toxin spectrum of various diphtheria poisons according to Ehrlich, in that the agglutinins are not absorbed in equal quantity by all bacteria, and a small per cent appropriate all that is available with the formation of flocculi in a clouded fluid.

Differences like the foregoing in the appearance of the agglutinated cultures, as well as other minor irregularities, made it necessary to determine, if possible, the influence of the bouillon used as a culture medium. It is well known that of the variable elements in the bouillon from fresh meat, the fermentescible muscle sugar is the most pronounced. It is likely to be nearly absent at times, especially in summer, and present in considerable amount in winter. Nay, the meat supply of different cities seems to vary somewhat in this respect, depending, probably, on the period of storage of the meat.

To imitate natural bouillon as closely as possible, a fermented and hence sugar-free bouillon was used, and to it different amounts of a sterile twenty per cent solution of dextrose were added with a calibrated pipette and the spermophile bacillus inoculated. After twenty-two hours' incubation, spermophile serum from rabbit No. 127 was used to determine the agglutinative limit. The following table gives all the necessary information in condensed form. The acidity is calculated in per cent of a normal solution. It will be noticed how the acidity of the cultures rises with an increase in the quantity of dextrose added. The activity of growth also varies from tube to tube, coming to a climax in the tube containing 0.16 per cent sugar, and falling as the quantity of sugar rises still higher. The agglutinative limit was determined by preparing dilutions of $\frac{1}{500}$ to $\frac{1}{8000}$ for each of the eight cultures. In the last column, the sign — indicates clumping seen with hand lens only. The signs +, ++, indicate clumping visible to the naked eye. This column shows that while differences exist, they are so slight that they might easily fall within the limits of error and that the sugar need not be regarded as an appreciable factor, in spite of the wide range of acidity of the bouillon (1.375 to 3.75 per cent of a normal solution) in which the bacilli were growing at the time the test was made.*

TABLE I. — SERUM OF RABBIT NO. 127, IMMUNIZED WITH SPERMOPHILE BACILLUS, TESTED WITH CULTURES OF THE SAME BACILLUS IN FERMENTED BOUILLON PLUS DEXTROSE.

TUBE NO.	Per cent of dextrose added.	Original acidity.	Acidity of cultures after 22 hours. (Per cent of normal solution.)	Estimated turbidity of culture fluid.	Agglutinative limit.
1	....	1.05 per cent	1.375 per cent.	100	8000 —
2	0.055	"	1.75 "	120	8000
3	0.11	"	1.875 "	190	8000 +
4	0.16	"	2.25 "	200	8000 + +
5	0.21	"	2.75 "	180	8000
6	0.26	"	3.5 "	160	8000 +
7	0.31	"	3.75 "	130	8000 —
8. Bouillon not fermented...		"	1.625 "	170	4000 —

III. INTERPRETATION OF RESULTS.

It has already been stated that the quantitative relation between the various cultures studied is best expressed by comparing the agglutinative limit of the cultures with reference to the same serum. Thus if typhoid and hog-cholera bacilli are acted upon by the same serum in dilutions respectively of $\frac{1}{20}$ and $\frac{1}{16000}$ in the same way, we may say that the agglutinin for the hog-cholera bacillus is present in eight hundred times the concentration as compared with the agglutinin for the typhoid bacillus.

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* According to Defalle (Annal. de l'Inst. Pasteur, 1902, p. 595), acetic acid in a dilution of 1/3600 clumps the typhoid bacillus. B. coli is clumped in a dilution of 1/1300. In the above experiment the difference between the lowest and highest acidity of the bouillon, calculated in terms of acetic acid, would represent a concentration of 1/2000; in terms of lactic acid, about 1/1000. There is no indication, however, that the acids produced during the multiplication of the bacteria played any part in the agglutination phenomenon.

In comparing the results, it would seem at first glance that the difference between a dilution of $\frac{1}{4000}$ and $\frac{1}{8000}$ is very great as compared with a dilution of $\frac{1}{25}$ and $\frac{1}{50}$. But, as a matter of fact, when we come to the limit of agglutination there is no more difference in effect between $\frac{1}{4000}$ and $\frac{1}{8000}$ than between $\frac{1}{25}$ and $\frac{1}{50}$, because in either case the higher dilution is one-half of the lower, and given a certain effect the rate of change in the dilutions reduced by one-half would be the same. This is the case so far as our experience goes. Hence the following series was finally chosen as most satisfactory: $\frac{1}{10}$, $\frac{1}{20}$, $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{4000}$, $\frac{1}{8000}$, $\frac{1}{16000}$, $\frac{1}{32000}$. The whole series of tests was not made in every case, especially in repetitions with the same serum where the limit was the main object sought. In many instances $\frac{1}{10}$ was omitted, owing to the large amount of serum required. Before proceeding to select animals which were to be immunized and become sources of the agglutinins, it was thought best to test the serum of a number of large and small domestic animals with the object of determining the presence of normal agglutinins. Among the species examined were several rabbits and guinea-pigs, two cats, two dogs, four pigs, one sheep, three cattle, and a number of horses yielding antitoxin, several immunized with typhoid bacilli, and one fresh one. Three specimens of placental blood from man were also included in the test.

In the cats, normal agglutinins were wholly absent. In the dogs, the limit of agglutination was $\frac{1}{10}$ or less. The serum from adult swine clumped the various cultures in dilutions of $\frac{1}{20}$ to $\frac{1}{200}$. A young pig yielded serum clumping in dilutions of $\frac{1}{10}$ to $\frac{1}{50}$. Sheep serum also clumped up to $\frac{1}{100}$. The three specimens of cattle serum clumped in dilutions of $\frac{1}{10}$ to $\frac{1}{100}$. The placental serum was practically devoid of agglutinins. Serum from the typhoid and diphtheria antitoxin horses clumped the various cultures in dilutions of $\frac{1}{20}$ to $\frac{1}{100}$ except *B. coli*. This was clumped in higher dilutions. Serum from unused horses clumped most cultures in dilutions of $\frac{1}{20}$ and *B. coli* VI. in dilutions of $\frac{1}{50}$.*

Guinea-pigs and rabbits were found, in general, free from more than traces of normal agglutinins. Preliminary test of the blood of such animals, when used for immunization, were not made. Since all the animals used were bred under supervision of one of us, it was certain that no infectious disease could mask the production of the specific agglutinins. The method pursued in comparing the various cultures was to immunize one or more animals toward each one of the cultures to be examined, and to test the blood of each toward all the cultures. This was carried out with both rabbits and guinea-pigs, but since the agglutinins did not appear in such concentration in the guinea-pigs inoculated with living cultures as in the rabbits, and since the results obtained with both species agree very closely, we have tabulated and plotted only the results obtained with rabbits.

The process of immunization consisted in the injection of both living and dead cultures. Whenever the living cultures were not virulent enough to prove fatal, they were used. At the same time cultures killed at 60° C. during thirty minutes were used for the least virulent cultures under the impression that larger amounts of the agglutinative substance could be introduced thereby, since the living bacilli of attenuated cultures multiply but feebly in the body of these animals.

In Table II. the various data concerning immunization are brought together as well as the agglutinative limits represented, for the sake of simplicity, by whole numbers instead of fractions. Thus 32,000 simply means that in a tube containing equal parts of culture fluid and normal salt solution and $\frac{1}{32000}$ volume of serum, the agglutination could still be detected after two or three hours, when compared with a

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* In all these tests the figures indicate the limit of agglutination as already stated not the complete clumping and subsidence of the bacteria.

TABLE II.

Culture used for immunization.	No. of rabbit.	Method of immunization.	Dose and number of injections.	B. coli vi.	Typhoid v.	Icteroides. (Orig.)	Gall Stone.	Hog cholera, Md.	Swine dysentery α.	Swine dysentery β.	Spermophile.	Guinea-pig disease α.	Guinea-pig disease β.	Miscellaneous Cultures.
B. coli vi. . .	**119**	Heated and Living cult.	5 separate injections.	**6,000**	10	10	< 10	20	10	10	10	10	—	(See Table III., of B. coli tests.) Fowl typhoid < 10.
B. coli x. . . .	**120**	Living cult.	3 separate injections.	< 20 (x.) **5,000**	(vii.) < 20	< 20	< 20	50	< 20	< 20	20	< 20	—	
B. coli xii. . .	**166**	" "	2 separate injections.	< 20 (xii.) **16,000**	< 20	< 20	< 20	< 20	< 20	—	< 20	< 20	—	
Typhoid v. . .	**124**	Heated cult.	1 agar slant 3 times.	10	**16,000**	20	100	10	50	—	100	100	—	B. coli ix. < 10. B. coli xi. 10. Dysentery (Shiga) < 10.
Icteroides . . . (Orig.)	**128**	" "	1 agar slant 3 times.	< 10	20	**8,000**	250	4,000	500	500	500	500	500	Hog cholera (non-motile) 500.
Gall Stone	**121**	" "	1 agar slant 3 times.	< 10	500	500	**8,000**	500	2,000	2,000	2,000	2,000	—	Shiga < 10.
	159	Living "	0.3 cc. and 1 cc. bouillon culture.	(xii.) 50	(vii.) 500	500	**8,000**	200	4,000	1,000	4,000	200	—	Typhoid iv. 100. Typhoid v. 100. Fowl typh. < 10.
Hog Cholera, Maryland . . .	**93**	Heated "	1 agar slant 4 times.	< 10 (xi.) 20	20	16,000	500	**32,000** (Mass.) 32,000	2,000	2,000	500	2,000	2,000	Hog cholera (non-motile) 500. Shiga < 10. Fowl typh. < 10.
Hog Cholera, Mass	**143**	Living "	0.1 cc. bouill. culture.	(xi.) 50	20	16,000	500	16,000 (Mass.) **16,000**	1,000	1,000	1,000	1,000	—	Hog cholera (non-motile) 500. Swine plague < 20.
Swine dysentery α . . .	**123**	Heated "	1 agar slant 4 times.	< 10	50	500	1,000	2,000	**8,000**	2,000	1,000	1,000	2,000	B. coli x. < 10. B. coli xi. < 20 Shiga < 10. Hog chol. (non-mot.) < 20.
	158	Living "	0.75 cc. bouill. culture.	—	50	100	200	200	**2,000**	100	500	500	—	
Sw. dys. β . .	**146**	" "	0.5 cc. bouill. culture.	—	20	50	100	250	500	**250**	250	500	—	
Spermophile,	**127**	Heated "	1 agar slant 4 times.	< 10 (xi.) 20	100	1,000	2,000	2,000	4,000	4,000	**4,000**	8,000	—	Shiga < 10. Fowl typh. 20.
	140	Living "	0.1 cc. bouill. culture.	(xi.) 50	20	100	100	100	500	8,000	**8,000**	16,000	8,000	
Guinea-pig disease α .	**188**	Heated "	1 agar slant 3 times.	—	50	1,000	2,000	2,000	2,000	8,000	16,000	**16,000**	8,000	Hog cholera, (non-mot.) > 20.
		Living "	0.75 cc. bouill. culture.	—	20	200	500	500	4,000	4,000	4,000	**4,000**	4,000	

control tube containing culture and salt solution, but no serum.

In order to make the results more uniform and to enable the reader to grasp them more readily, they were plotted by erecting ordinates. The ordinate of the culture agglutinating in the highest dilution was made one hundred: the others were calculated accordingly. In all but one case, the highest ordinate corresponds to the culture used for immunization. The exception is the serum of the spermophile rabbit. This serum agglutinated the culture, guinea-pig disease *a*, in higher dilution than its own culture; hence in this case the fifty ordinate should be looked upon as the standard.

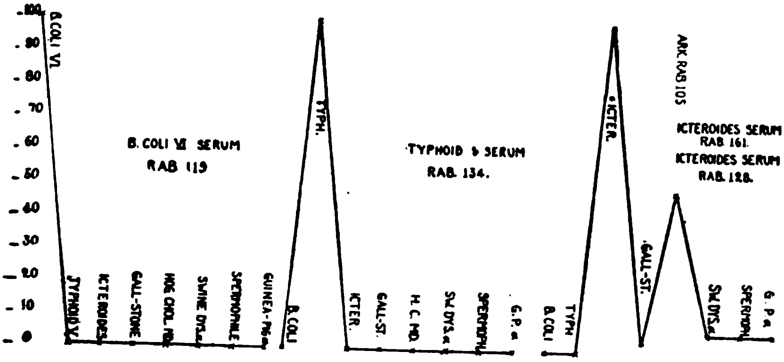

FIGURE I.

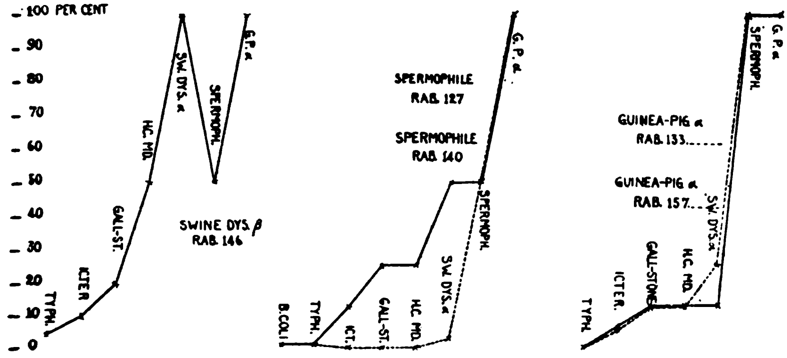

FIGURE 2.

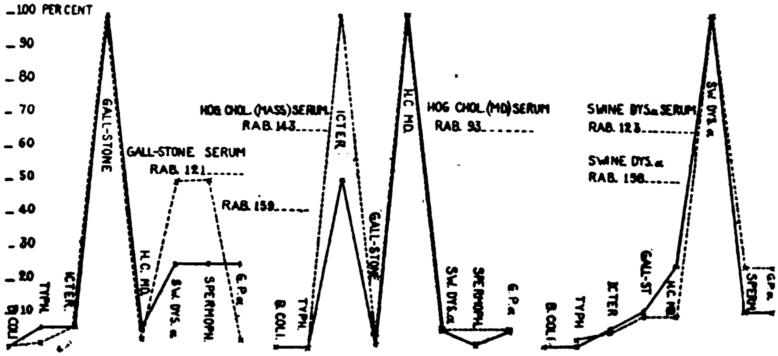

FIGURE 3.

In order to make the results more graphic and to bring out the very low percentages of the agglutinative limits of some cultures, the tops of the ordinates were connected by lines so as to yield a "curve."

Among the various deductions which might be made from the tabulated results, the following seem well founded:

1. The persistence of the agglutinins in undiminished strength for months in the drawn serum. This has been noticed by many others. In our own studies, the serum, to which no antiseptic was added, was kept in test tubes, plugged with cotton wool, in the refrigerator. Repeated tests made with the same serum after an interval of months yielded the same results.*

2. The persistence of specific agglutinative relationships after years of artificial cultivation. Thus the various hog-cholera bacilli ranged in age from sixteen to three years, yet their close relationship was manifested in all the tests.

3. The most virulent cultures towards rabbits yielded a

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* The persistence of the agglutinins in dried blood is illustrated by the following experiment:

Aug. 10, 1898. Several drops of blood of an immunized rabbit were dried on filter paper in the air and preserved in a corked bottle.

Aug. 17, 1900. The paper was soaked in salt solution so as to make an approximate dilution of 1/50. Clumps formed at once with the guinea-pig bacillus α, the immunizing culture, in 1/100 dilution. Prompt clumping was also obtained in a dilution of 1/400. No other dilutions were tried. The original clumping power was about 1/1000.

serum of greatest clumping power towards themselves, as shown by hog cholera, Maryland and Massachusetts. When living cultures are used, the clumping power is greatest in those sera produced by the most virulent bacilli, evidently because the multiplication and distribution in the body is more extensive. (Compare Nos. 140, 158, and 146 as regards dosage and agglutinative results.)

4. In general, cultures killed at 60° C., if injected in sufficient quantity, yield as concentrated an agglutinin as living cultures.

5. Bacteria isolated from well-defined types of disease, especially those known to invade the blood regularly, are clumped in nearly the same dilution. This was true of four different cultures of the typhoid bacillus, of five different cultures of the hog-cholera bacillus from widely separated parts of the United States, and *B. icteroides* original and *B. icteroides* Santiago. The two paracolon bacilli (gall stone and gall bladder) also closely agreed in all tests to which they were submitted. This harmonizes with the inference that they were really identical. In the same way the two stocks of guinea-pig disease *a* and *β* agreed.

6. The bacteria just referred to belong not only to the same host, but they are morphologically and biologically identical and induce the same group of symptoms. There are, however, agglutinative relationships which overstep these narrow limits. Perhaps the most striking and persistently uniform relationship manifesting itself, not only in the serum of rabbits, but also in that of several guinea-pigs, is that between *B. icteroides* and hog cholera *a*, as shown both in the figures and curves. These two groups cannot be separated by the agglutination test, for the reciprocal action of the specific sera was the same. At the same time the virulence of *B. icteroides*, as tried upon rabbits, was much lower than that of hog cholera *a*.*

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* The close relation existing between these two bacilli, as regards pathogenic properties, was demonstrated by Walter Reed and James Carroll several years ago. See Medical News, April 29 and Sept. 9, 1899, and Journ. Exper. Med., 1900, v, pp. 216–270. In view of these many points of resemblance, it is of interest to inquire whether *B. icteroides* is a human or a porcine parasite.

A similar close relation subsists between the sphermophile and the guinea-pig bacilli; in fact, in several instances the spermophile serum agglutinated the guinea-pig bacillus in higher dilution than its own bacillus. This result may, perhaps, be found to point the way to the use of the guinea-pig bacillus in the extermination of sphermophiles.*

7. The table furthermore demonstrates a general though variable relationship of all the bacilli producing gas in dextrose only, with the general formula $\frac{H}{CO_2} = \frac{2}{1}$, and not producing indol. It shows that the true B. coli (pig) stands quite apart from these. Similarly, the dysentery bacillus (Shiga) remains unaffected. The typhoid bacillus shows a slight relationship to the group in that it is uniformly clumped in low dilutions of the serum which clumps the others in dilutions of 1,000 and above.

8. The statement has been made, notably by Durham,[6] that agglutination toward related bacteria may not appear unless the agglutinating power of the blood be driven very high. This is in so far true as the low agglutinative value toward other bacteria may be overlooked. But it is, nevertheless, present. A comparison of rabbit No. 123 with No. 158, and of No. 133 with No. 157 (Table II.), indicates a certain proportionality in the production of agglutinins towards bacteria, even though the actual quantities produced differ greatly. (See curves.) It is, however, very desirable to force the agglutinins to such a concentration that dilutions below $\frac{1}{20}$ need not be used, since the more concentrated dilutions of serum may contain unknown anti-bodies which may modify the reaction, or they may contain normal agglutinins in sufficient amount to become recognizable.

9. A careful study of the table will reveal certain irregularities in the results. Thus while *B. icteroides* and hog

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* Similar agglutinative affinities of bacilli apparently not at all related are reported by C. Sternberg.[13] He found three bacilli in water which produced gas (in which sugar?) which had no coagulating effect on milk, and were practically non-pathogenic in animals, but which were agglutinated by typhoid serum in dilutions of 1/1000. Two similar cultures were found in the Institute collection. Rabbits treated with each of these water bacilli yielded a serum which agglutinated the typhoid bacillus in nearly the same dilution. A similar relationship was established by typhoid serum from the horse.

cholera, Maryland, agree closely in the reciprocal effect of their respective sera, yet when tested with other sera we find occasional differences. Thus the serum of swine dysentery *a* acts upon *B. icteroides* and hog cholera in the ratio of 500 : 2,000 and 100 : 200. The serum of swine dysentery *β* acts upon the same in the ratio of 50 : 250.

Similarly, swine dysentery *a* and *β*, which are very closely related biologically, are acted upon with nearly the same energy by various immune sera. Yet the serum of swine dysentery *a* (rabbit 158) acts upon *a* and *β* in the ratio of 2,000 : 100; the serum of spermophile (rabbit 140) upon the same in the ratio of 500 : 8,000. Possibly the differences that exist between such bacilli as *B. icteroides* and *B. choleræ suis a*, which have mutually identical agglutinative affinities, may best be determined by treating them side by side with immune sera of other related bacilli.

10. Returning to our thesis with which we started, *i.e.*, whether agglutinative relationships are called forth by the host by augmenting or reducing the original capacities of the parasite, we may formulate our deductions in this way, that undoubtedly the host influences the parasite, and that given species of like origin, as indicated by morphological and physiological characters and living in the same relation with the tissues of the same host-species, identical agglutinative tendencies may be predicted. Where these conditions are not fulfilled, no identity in this regard need be anticipated. Thus the typhoid, icteroides, and gallstone bacilli are all from man, but the typhoid bacillus is probably of a different origin and the agglutinative relation is distant. Whereas the other two are more nearly akin and their agglutinative relation is much closer.

Perhaps the best illustration is afforded by the various cultures from pigs. We have three sets of cultures to consider, the true hog-cholera bacilli, the bacilli designated swine dysentery, and the colon bacilli. The two former differ from one another in that the swine dysentery bacilli grow much more vigorously and are far less pathogenic than the hog-cholera bacilli. Furthermore, the latter invade the blood and may be found in various organs, especially the spleen; the former are rarely found in the spleen or liver and are then accidentally escaped individuals, perhaps. The true colon bacilli live only in the intestines. It seems from our results that besides general agreement in morphological and biological characters, there must be an agreement as to their habitat in the body, and that bacilli limited in the main to the intestine and its walls are not identical with truly invasive forms. In other words, the parasitic adaptation which also determines the locus or habitat of any bacillus in the body may be of great influence in adjusting agglutinative relationships.

A very interesting illustration of agglutinative affinities is that presented by a non-motile hog-cholera bacillus described by one of us some years ago.[6] This stands in a definite relation to the motile bacillus *a* and to *B. icteroides* in presence of specific sera. Since the agglutinative phenomena of this non-motile variety present other important characters, this subject is reserved for another communication.

It will thus be impossible by agglutination to trace the source of pathogenic bacteria in foreign hosts, such as in meat infection or meat poisoning, unless we take into consideration other characters as well, and test the patient's serum towards a great variety of bacteria, since several races or varieties may come from the same host. This inference is in general confirmed by the observations made by B. Fischer.[7] Fischer tested a number of cultures obtained by himself and others from human beings suffering from meat poisoning, and from animals from which such meat was derived. His results may be summarized as follows:

Immune sera were prepared by inoculating goats with two different stocks of *B. enteriditis* from the cow. The agglutinative affinities in presence of these two sera varied considerably with the cultures tested.

Thus the two stocks used for immunization, a third stock from a case of mastitis in the cow, Van Ermengen's culture of calf diarrhea (Morseele, 1892), his culture of meat poison bacilli (Ghent, 1895) from sausages of pork and smoked beef, and Gärtner's *B. enteriditis* were agglutinated in a dilution of $\frac{1}{40000}$. On the other hand, Känsche's cow bacillus and Günther's bacillus from swine were agglutinated in dilutions of $\frac{1}{40}$, while swinepest, mouse typhus, and Basenau's *B. morbificans bovis*, an indol-producer, were not agglutinated at all.

In this summary we note that Känsche's cow bacillus fails to respond with the other cow bacilli. *B. morbificans bovis* may be excluded, since according to Fischer it produced indol. Fischer also states that the goat's (enteriditis) serum agglutinated typhoid bacilli in $\frac{1}{50}$ — $\frac{1}{100}$ dilutions, and that, conversely, a typhoid serum which agglutinated typhoid bacilli in $\frac{1}{5000}$ dilutions agglutinated his enteriditis stock in $\frac{1}{50}$ dilutions. This slight reciprocal relation between typhoid bacilli and the pathogenic colon derivatives agrees with our own results.

IV. THE AGGLUTINATIVE AFFINITIES OF B. COLI AND ITS ALLIES.

The group of colon bacilli occupies a somewhat different position towards our subject than the bacilli considered thus far. The latter, with the possible exception of the two swine dysentery bacilli *a* and *β*, are true invasive bacteria; whereas the colon bacilli vegetate in the digestive tract. This is open to colon bacilli from a great variety of sources, the vehicles in man being more particularly water and milk. We do not yet know whether the colon bacilli, so numerous in the large intestine of domestic animals during health, are true parasites of the individual or the species or not. Even if they should be found to be parasites, each adapted to a species or race, changes in the condition of the mucous membrane in disease may temporarily suppress such normal flora and substitute for it colon bacilli adapted to such pathological products as muco-pus, serous exudation, etc. The conditions are thus quite complicated, and the results of recent investigations reflect this complexity.

Studies in this group have been conducted by a number of observers recently on a very extensive scale, with the view of determining what relation, if any, exists between cultures from different sources. It would be impossible to analyze and justly estimate the significance of this work at this time, and we wish simply to call attention to a few points as introductory to the few cultures examined by us.

Emphasis has been laid in the preceding pages on the necessity for certain preliminary uniform conditions in the study of related cultures. These are identity of habitat and of biological characters. These conditions are realized only in part in the papers published.

Sidney Wolf[8] isolated from a suppurating inguinal hernia a motile bacillus having all the characters of *B. coli*, save that it failed to produce gas in two per cent dextrose media. Surprise is expressed by the writer that while the blood of the patient agglutinates this bacillus in dilutions of $\frac{1}{100}$, it does not act upon a colon bacillus from the feces of the same patient. Wolf probably had here two quite different organisms to deal with, as the absence of fermentative powers shows.

Jatta[9] found in the same stools several *B. coli* behaving differently toward one another's serum. Detailed studies of the biological characters of a number of colon bacilli from different sources are given, but the fermentative characters only very imperfectly.

H. L. Smith[13] isolated a number of cultures of *B. coli* from the stools of the same individual, which reacted alike towards the serum produced with one of them. Cultures from other sources reacted negatively.

Rothberger[10] studied forty different stocks from various sources and found no definite relationship between them, as regards agglutination.

Radzievsky[11] endeavored to obtain uniformity as regards habitat by studying sixty-four cultures isolated from the stools (or rectum) of one and the same individual. He also is able to establish groups acted upon by serum produced by some one of them, but there is no uniformity. Though much time is devoted by both Rothberger and Radzievsky upon the cultural characters of the bacilli studied by them, yet the division is unsatisfactory because the fermentative properties are not sharply defined. Thus Rothberger does not even state what sugar is used by him. Radzievsky uses peptone water and lactose to determine gas formation.

These quotations indicate that much is still to be learned concerning the bearing of agglutination upon host, habitat in the host, and biological characters of colon bacilli. Especially is it desirable to study in detail the agglutinative affinities of varieties of colon bacilli from some animal like the pig, in which the colon flora of the large intestine is so much more uniform and pronounced than in man. The following study is merely a beginning, since the number of cultures used is very limited. The various stocks of *B. coli* used in our tests were isolated by one of us from pigs, with one exception. They present various interesting aspects which must be considered in connection with the results of the agglutinative tests.

B. coli VI. was isolated in 1900 from a pig from which swine dysentery *a* was obtained. It was regarded as a typical colon bacillus as the table clearly shows. In 1902 it was replated and a non-motile variant VI.b separated from it, whose surface colonies were at the time somewhat thicker and more opaque than the other colonies. Several months later the culture VI.b was again plated, and this time the surface colonies were all of the very thin expanding type. A similar tendency of individuals in the same culture to vary is shown by Bac. XI. and XII. These were isolated as one culture from the spleen of a pig (swine dysentery) in 1902. Such cultures are as a rule pure from the start, but the gelatine rolls, prepared to study the culture in detail, showed two types of surface colonies, one like most colon bacilli, a thin, translucent, bluish expansion, the other a thicker and more opaque, white, convex disc, suggesting drops of mucus. When the tube was placed upright, the colonies tended to flow a little down the gelatine layer. The latter were few in number. Sub-cultures and the repetition of gelatine rolls three times in succession finally eliminated the thicker colony. When, however, both were carefully studied side by side, it was found that there were no differences between XI. and XII., excepting in this, that XII. contained very few motile bacilli and produced more capsular substance as suggested by the tendency of the surface colonies to flow slightly.

The explanation which agrees most closely with these facts is, that the two bacilli XI. and XII. are really identical, derived from the same original stock, bacillus XII. being a variant in the direction of non-motility and with a tendency to produce more capsular substance. Bacillus XII. at first suggested *B. lactis aërogenes*, but several characters opposed this designation: the very marked indol production, strongly offensive odor, and the occasional appearance of motile bacilli. More recently B. XII. was again plated, and this time the colonies were wholly different. The glistening drop-like surface colony was wholly replaced by the thin expansion. Hence we may safely assume that Bacillus XI. and XII. are identical, but that the stock tends to vary sufficiently to modify the appearance of the surface colonies as well as the relative motility from time to time. We must thus consider VI.a and VI.b as identical; also XI. and XII. The tendency of these organisms to vary in their motility may lead to more or less difference in the appearance of surface colonies. Variations in the production of capsular substance and in its viscidity, which occasionally occur during prolonged artificial culivation, are also likely to modify the configuration of surface colonies. The biochemical characters, however, do not appear to change appreciably.

The series of colon cultures in the table is interesting in another particular. It represents gradations from the true colon bacillus to the pathogenic forms. Thus Bacilli VI., VII., and VIII. agree in the main. Bacilli IX. and X. have taken a step toward a more pronounced parasitism in that they have lost the power to act upon saccharose. Bacilli XI. and XII. are still farther along in this direction and unique in many respects. They have nearly lost the power to produce gas in lactose bouillon, as only a bubble usually appears, but acid production still survives. It is, however, impaired, and the acid accumulates quite slowly. As a result of this impairment, milk remains at first unchanged. After several days a curd forms below, the milk becomes thicker throughout, and after a week the entire fluid is solid. In some lots of milk boiling was necessary to bring about coagulation. The indol production has not been lost and the odor of cultures is still offensive. The next members in this series towards the typical hog-cholera bacillus are the swine dysentery α and β, which have lost the power to act on lactose wholly, and which do not produce indol. The agglutinative tests made with these varieties of *B. coli* are summarized in Table III. The action of the immune serum upon the pathogenic group will be found in Table II. A glance at the table shows that the agglutinative relationship runs nearly parallel with the biological characters and with the host. Thus immune serum of typical *B. coli* VI. agglutinates VIII. markedly, but not VII. These three have the same biological characters, but VII. is from a fowl. Immune serum VI. shows a slight relation to the type IX.–X., but not to XI.–XII. Immune serum X. acts strongly upon IX., quite strongly upon VIII., but not on VI. and XII. Lastly, serum XII. fails to act upon any of the others. The only exception to be noticed is the action of serum X. upon VIII. where only a feeble effect was anticipated.

Table II. shows that none of the pathogenic derivatives of *B. coli* are appreciably affected by the immune sera of the three varieties of *B. coli.* The same is true of the typhoid bacillus.

To explain the more or less specific agglutinative reaction of these closely related forms we may assume with Durham that each bacillus possesses a number of agglutinative bodies or receptors in Ehrlich's sense which differ both qualitatively and quantitatively in the different races of bacilli. There are, however, even after taking into account the existence of a number of different affinities for each variety, still certain discrepancies which may, perhaps, be eliminated by a more careful technique and a larger series of animals.

These observations on B. coli, while they do not prove our thesis, do not militate against it. It should be borne in

TABLE III. — AGGLUTINATION TESTS WITH VARIETIES OF B. COLI.

B. coli. No.	Source.	Motility in 24-hr. bouillon cultures.	Bouillon in fermentation tubes with 1%.			Milk Coagulation in	Gelatine plates or rolls. Surface colonies.	Indol production.	Agglutinative relationship as determined by blood-serum of			
			Dextrose.	Lactose.	Saccharose.				Rabbit treated with B. Coli VI.a. and b.	Rabbit treated with B. coli X.	Rabbit treated with B. coli XII.	Guinea-pig treated with B. coli XII.
VI.a. VI.b.	Liver of pig (swine dysentery), June, 1900.	50% of bacilli non-motile.	Typical B. coli fermentation.	Typical B. coli fermentation.	Slow gas and acid production	36 to 48 hrs.	VI.a. Delicate bluish expansions. VI.b. Slightly thicker	Marked.	VI.a, **6,000** VI.b, **2,000**	Less than 20	Less than 20 Less than 20	Less than 20 Less than 20
VII.	Liver of fowl (fowl typhoid).	Very few motile bacilli.	Typical B. coli fermentation.	Typical B. coli fermentation.	Slow gas and acid production	36 to 48 hrs.	Delicate expansions.	"	20	Less than 20	Less than 20	Less than 20
VIII.	Liver of pig (swine dysentery), Apr., 1902.	30% to 50% motile.	Typical B. coli fermentation.	Typical B. coli fermentation.	Slow gas and acid production	24 to 36 hrs.	" "	"	1,000	1,000	Less than 20	Less than 20
IX.	Small intestine of pig, June, 1902.	20% to 30% motile.	Typical B. coli fermentation.	Typical B. coli fermentation.	No acid or gas production.	24 to 36 hrs.	" "	"	200	2,000	Less than 20	Less than 20
X.	Mesenteric gland of pig (swine dysentery), May, 1902	20% to 30% motile.	Typical B. coli fermentation.	Typical B. coli fermentation.	No acid or gas production	24 to 36 hrs.	" "	"	200	**4,000**	Less than 20	Less than 20
XI.	Spleen of pig, May, 1902.	50% to 75% motile.	Typical B. coli fermentation.	Slow acid production no gas.	No acid or gas production.	7 to 8 days.	" "	"	Less than 20	......	....	....
XII.	Spleen of pig, May, 1902.	10% or less motile.	Typical B. coli fermentation.	Slow acid production no gas.	No acid or gas production.	7 to 8 days.	Fleshy convex pearly colonies tend to run down sides of gelatine roll.	"	Less than 20	Less than 20	**16,000**	**500**

mind, and it has already been referred to, that adaptation to a given host such as the pig implies a number of degrees of adaptation. Thus B. coli may be adapted to the food-remains in the large intestine, to the mucus on the surface of the epithelium, to the contents of the tubules and larger flask-shaped mucous glands. Races may exist which live only in these glands. Others may be able to subsist in the follicles, similar to the bacilli found by Ribbert in the follicles of the rabbit's appendix. With this progressive adaptation are associated modifications of biological characters which, most likely, involve agglutinative capacities as well.*

V. CAN AGGLUTINATION BE MODIFIED BY PASSAGES THROUGH ANIMALS?

The hypothesis advanced that the host may impress specific agglutinative characters upon parasitic bacteria by stimulating the development of some and suppressing others, until a definite agglutinative formula has been reached by which the same pathogenic organism may be identified the world over, is not one capable of ready proof or disproof, because the adaptation is probably a very slow process and cannot be imitated in the laboratory.

Nevertheless a trial was made with a virulent hog-cholera bacillus (Arkansas) upon rabbits and guinea-pigs. A rabbit was inoculated with a minimum fatal dose and after death, which occurs in seven to nine days, a bouillon culture was prepared from the animal, and a second inoculated on the following day. Fourteen rabbits were used in series, the experiment extending over a period of five months. It will be seen that there must have been ample multiplication in the bodies of rabbits, for the subcutaneous dose, starting with 0.1 cc. of a bouillon culture, was reduced with each succeeding animal, the last dose being 0.00001 cc.

A similar undertaking was carried through with guinea-pigs, for it was supposed that the gap between the final guinea-pig stock and the final rabbit stock might be wide enough to be appreciated in the agglutination tests. Fourteen guinea-pigs were used in the series, and the time required was about six months. The guinea-pig is much less susceptible to the hog-cholera bacillus *a* and doses of 0.3 to 0.4 cc. of a twenty-four-hour bouillon culture were not regularly fatal. Those guinea-pigs that survived were chloroformed after two or three weeks and the bacillus recovered by adding bits of spleen or liver to bouillon and agar. One of us had previously shown that in rabbits the bacilli can still be obtained from spleen, liver, or kidney three or four weeks after the high temperature and all other signs of illness had disappeared, by adding bits of such organs to culture media.[14]

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* Recently Escherich and his pupils have gone a step farther and affirmed not merely a race but an "individual reaction" on the part of *B. coli* from different human beings. This advanced position is not quite sustained in our results with *B. coli* from the pig, for all the cultures used, except XI., XII. were from different pigs. (See literature 13.)

A careful comparative test of these passage bacilli and the original with immune serum did not reveal any differences attributable to the passages. In other words, no decided modification of the agglutination phenomena had taken place.

An impartial analysis of this and similar experiments suggests certain difficulties which may stand in the way of change.

1. The hog-cholera bacillus *a* may be so specialized as to fail in further adaptation, and such a modification as is looked for may need an undifferentiated stock which gradually acquires specific properties in different hosts.

2. In case of a bacillus which already possesses a maximum virulence toward a species, as does the one used towards rabbits, any modification may be impossible. Such modification may need a highly resistant host.

Whichever explanation may prove the true one, it is evident that the specific agglutination functions are slowly acquired and tenaciously held.*

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* After the completion of the manuscript, F. Meyer's paper on the agglutination of streptococci (Deutsche Med. Wochenschrift, 1902, p. 751) was found to contain some facts bearing upon this subject. Meyer states that a serum of Aronson's prepared with streptococci continually passed through mice failed to agglutinate human streptococci. When, however, the latter were passed through a series of mice (how many is not stated) they were agglutinated by Aronson's serum. If these experiments should be confirmed, they would show a much greater plasticity of the streptococci than is found in the hog-cholera group of bacilli.

VI. WHAT RELATION DO PARACOLON BACILLI FOUND IN MAN BEAR TO THOSE OF ANIMAL ORIGIN?

Of the general group of bacilli to which the gall-stone and gall-bladder bacilli of our series belong, are those denominated paracolon or paratyphoid bacilli, which have been isolated from the human subject under various pathological conditions. Achard and Bensaude[15] were the first to call attention to this group of bacilli. Of those discovered subsequently, a good resumé is given by Libman.[16] Among the group of cases of disease caused by these bacilli, those that have aroused, perhaps, the most interest are such as simulate typhoid fever, but whose sera fail to give positive clump reactions with typhoid bacilli. The most notable cases described are those of Gwyn,[17] Cushing,[18] Schottmüller,[19] Kurth,[20] Buxton and Coleman,[21] and Berg and Libman.[22]

The meat-poison group of bacilli isolated by various observers from man are probably all derived from animal sources, more particularly cattle and swine.

The relation which the latter group bears to the former is naturally a question of great interest, as also the one whether there is any true paracolon or paratyphoid bacillus which is responsible for a definite set of symptoms in man simulating typhoid fever. The uniformity in the agglutinative characters of such well-defined species as the typhoid bacillus and hog-cholera *a* would naturally lead us to assume that a like intimate relation should exist between the various bacilli producing a typhoid-like affection. Various efforts have been made to group or classify the many cultures, both of the meat posion and paracolon group, either by endeavoring to establish relationship between the two groups or between individuals of the same group. Among those who have made more or less exhaustive comparisons are Durham,[6] Cushing,[18] and Buxton.[23]

A glance at the table of agglutination affinities given by Buxton suggests a grouping into bacteria of animal origin and those belonging to the human subject, together with some intermediate forms. But there is as yet no evidence which would enable us to create a distinct pathogenic species of paracolon or paratyphoid bacilli, because the agglutination tests are still conflicting or, at least, not worked out with sufficient detail.

In the endeavor to group bacteria so closely related as are these under consideration, the question will always arise as to the relative value of the various biological tests. It seems that perhaps too much weight is given to reactions of quantitative value merely, of a little more or less, such as, for instance, the production of a little more or a little less gas in the presence of sugar of a certain kind. Perhaps the most satisfactory grouping is one which uses only constant qualitative differences, leaving minor quantitative ones to adjust themselves.

Of the qualitative tests of fundamental importance is the formation or non-formation of gas or acids, or both in presence of certain sugars, and the presence or absence of indol. With these tests in view a grouping is readily effected and possible transition forms are easily placed. The agglutination tests should endeavor to establish a definite relation between the agglutinative limits, and if this relation should prove to be unity in the case of bacilli isolated from the same species, identity may be inferred even when other properties, such as motility or fermentative capacities, are relatively weakened or exalted.*

CONCLUSIONS.

1. There exist agglutination relationships between the pathogenic groups of bacilli which ferment dextrose as given in the text. This relationship is not brought out clearly unless the agglutinative limit of the various cultures is worked out with a serum agglutinating its specific bacillus in dilutions of one to one thousand and above.

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* It is not improbable that the paracolon bacilli are a miscellaneous group from various sources striving to become parasites in the human subject, but not yet sufficiently adapted either to produce a distinct type of disease or to have acquired the same agglutination affinities.

2. Agglutinative characters are probably modified quantitatively when the same bacillus becomes parasitic upon different hosts, some being weakened or suppressed, others augmented.

3. Close agglutinative affinities may be predicted from close biological and pathogenic relationships.

4. Minor cultural differences involving membrane formation in bouillon and differences in the appearance of surface colonies do not exclude close agglutinative affinities.

5. It is not possible to trace the members of this group attacking man back to the animal species from which they may have come, by agglutinative tests alone. This may, perhaps, be done in combination with cultural and pathogenic tests after the various races parasitic upon different animal species shall have been more thoroughly identified.

6. It appears that closely related bacteria vegetating on mucous membranes may vary considerably in their agglutinative relationships,[1] differing in this regard from the more invasive species whose varieties or races are more homogeneous.

7. Of the pathogenic cultures examined, *B. icteroides* and the hog-cholera bacillus *a* on the one hand, and spermophile and guinea-pig disease *a* (and *β*) possess nearly identical agglutinative properties.

8. The typhoid bacillus shows slight agglutinative affinities with the group described under I., above. The typical colon bacillus shows none.

9. Identical biochemical properties of *B. coli* from the same host are associated with close agglutination affinities.

10. Two races of bacilli, whose sera agglutinate both races the same quantitatively, may be acted upon differently by the serum of a third race.

11. Guinea-pigs and rabbits immunized in the same way with the same culture yield sera whose agglutinins are the same qualitatively. Exact quantitative uniformity may or may not be obtained and depends upon the relative susceptibility of the animal and the use of living or dead cultures.

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[1] This is suggested, furthermore, by the study of the agglutination of diphtheria and dysentery bacilli by various investigators.

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ACTINOMYCOSIS OF THE CENTRAL NERVOUS SYSTEM, WITH THE REPORT OF A CASE DUE TO AN UNIDENTIFIED MEMBER OF THE ACTINOMYCES GROUP.*

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The observation of an apparently primary abcess of the brain caused by a branching, intertwining, thread-like organism similar to those recently found in a variety of lesions, and commonly called streptothrix, but properly classed under the genus Actinomyces, led to a review of the literature of actinomycosis of the central nervous system.

Including mine, eighteen cases were found, and of these the nervous system was apparently affected primarily in five, and secondarily in thirteen. On comparison many of these are very similar to each other and to our own.

It is my intention in the present article to state the salient features of the cases in the literature, report my own case, call attention to the identity of so-called Streptothrix with Actinomyces and, finally, summarize our knowledge on the subject.

I. PRIMARY ACTINOMYCOSIS OF THE CENTRAL NERVOUS SYSTEM.

The first recorded is that of O. Bollinger[1] (1887), who reported the case of a woman aged twenty-six years, whose illness began about one year before death with headache, soon after which there was paralysis of left N. abducens. Six months later there was paralysis, with eczema, of the forearms, and about this time she gave birth to a vigorous boy. Headache persisted, and nineteen days before death there was double vision and changes were made out in the left retina. Death occurred in coma after convulsions. Diagnosis,

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brain tumor. The lateral ventricles were enormously dilated, and the foramen of Monro and the third ventricle were distended by a growth the size of a hazelnut, with smooth surfaces, and of a bluish gray color. On section a mucoid, sticky fluid escaped. Microscopically the mass was composed of granulation tissue containing numerous typical colonies of actinomyces. No other focus of actinomycosis was to be found in the body. The woman is said to have had bad teeth.

Almquist[2] (1890) reported the case of an artilleryman who died after a two weeks' illness with cerebro-spinal meningitis. The autopsy was made twenty hours after death. Plate cultures made from the base of the brain and from the lateral ventricles gave colonies of a large micrococcus, B. proteus, and a single colony of a streptothrix. This organism grew well on gelatine, forming rather large convex hemispherical colonies and causing liquefaction of the medium. On agar there was a rapid luxuriant growth, with larger and smaller white crusts. In broth there was a flaky growth, with a thin surface crust. Microscopically there was a mycelium of unjoined threads with true and frequent branching. The threads averaged 0.5 μ. in diameter and sometimes reached 100 μ. in length. Air hyphæ which broke up into small cubical or oval cells appeared on the white crusts. Both cells and threads stained lightly with the aniline dyes. No mention is made of finding this organism in the pus or sections of the meninges, or of animal experiments. Absence of microscopical proof of the presence of this organism in the lesions and the fact that a single colony was obtained in the cultures lend strong probability to the natural inference of air contamination. I am, therefore, very strongly inclined to question the propriety of including this case.

In 1895 Ferré et Foquet[3] reported the case of abscess of the brain of a man who during life had epileptiform crises. From the description the brain abscess was probably primary. From the pus they obtained a "streptothrix" in pure culture. The organism grew well on the various media, but best on potato. Microscopically it was made up of ramifying filaments with knob-like terminations. The organism stained by Gram and was not pathogenic for guinea-pigs. On subdural inoculation of a rabbit there was general diffusion of the parasite in the organs without reaction or the formation of pseudo tubercles.

Musser, Pearce, and Gwyn[4] in 1901 reported a case of probably primary brain abscess in a man twenty-four years old. "At a point just posterior to the upper portion of the fissure of Rolando is an area three centimeters in diameter which is fluctuant, grayish yellow in color." The side of the brain affected is not mentioned. On section at this point there was found an abscess 3.5 cm. in diameter, containing thick partly chocolate-colored and partly grayish yellow fluid of the consistency of oil. No granules were made out. The walls of the cavity were greenish gray in color and soft and neurotic. The brain tissue about the abscess was softened and of a yellow color. The pus had a foul odor. (The striking resemblance to the abscess in my case will be noted.) The rest of the brain was normal. The ears and the bones of the skull appeared normal. The other organs could not be examined. In the pus of the brain abscess after search there was found a large ball-like tangled mass made up of long irregularly staining filaments. There was some suggestion of branching of the periphery of the mass. Histological examination of the abscess wall showed a large mass of radiating threads, which at the periphery were long and thin and stained irregularly. The whole had the appearance of an actinomyces colony. Clubs and rosettes could not be demonstrated. No tubercle bacilli could be found. Aerobic and anaerobic cultures and animal inoculations (guinea-pigs) were negative.

J. C.—Male, white, aged fifty-two years, was admitted to the medical wards, service of Dr. Edward F. Cushing, of the Lakeside Hospital, April 21, 1902. The patient had been an orderly in the hospital for a number of years and had always been well until his present illness. Several days before his illness he had complained of not feeling well, and for two days before admission to the ward he had not reported for work. On the morning of April twenty-first he was found in his room in a condition of stupor. He was very drowsy, but on being aroused could answer questions and complained of pain in his right eye. The conjunctiva of this eye was reddened, the other pupil was markedly dilated. The left eye and pupil appeared normal. The patient could move his hands and arms and there was no evidence of paralysis. His eyes were examined by Dr. Millikin, who found nothing abnormal. Physical examination disclosed nothing further except varicosity of the veins of the left leg. Ulceration of the leg is not mentioned in the clinical history and was therefore probably not present. On April twenty-second his condition was much the same, but questions were answered with difficulty and stupidly. The right pupil was dilated and did not react to light. Breathing was regular and somewhat accelerated. The pulse was full sixty-four to the minute. Physical examination of the thorax and abdomen was negative. The knee jerks on both sides were much increased. On the left side there was a marked Babinski reflex. The patient could move his upper and lower extremities, but there was marked rigidity of the muscles of the left arm and to a less degree those of the left leg. The temperature ranged between 100° to 101.5° F. There was involuntary micturition and defecation. On April twenty-third the note made records that the patient continued throughout the preceding day in a comatose state, and during the night the coma deepened. At times he was quite restless and complained of pain in the right side of the head. To-day there was deep coma from which he could not be aroused. The right pupil was still widely dilated; the reflexes were the same as yesterday. There was now rigidity of the right arm and leg, with paralysis of the left arm and leg. Breathing was stertorous, but not especially rapid. The pulse was strong and eighty to the minute. The leucocyte count showed twelve hundred per cubic millimeter. There was no discharge from the ears, the membranes appeared normal. He was transferred to the surgical service of Dr. Dudley P.

Allen, who trephined over the motor area of the right side. There was distinct bulging of the brain when the dura was cut through. The meningeal vessels were markedly congested, but no hemorrhage and meningitis, and no evidence of brain abscess were found. The rigidity of the arms became less marked after incision of the dura. The patient died at five-forty the afternoon of April twenty-third. Autopsy six hours after death. The body was fairly well built and nourished, and 170 cm. in length. Rigor mortis was present. The right side of the head was shaved and over the right parietal region there was a semicircular wound closed with sutures. The right pupil was somewhat larger than the left, which was of ordinary size. The external surface of the left leg was the seat of a large varicose ulcer. Near this there were a number of scars of former ulcers. No other scars were to be seen. The head was of ordinary size and shape. The wound above referred to was healthy in appearance and free from pus. The skull was of ordinary thickness and appearance. There was no softening. The dura at and near the seat of the operation was markedly congested. The brain and pia-arachnoid bulged slightly at the opening. The pia-arachnoid over a considerable area was congested and in a large vein just beneath the wound contained a dark red thrombus. On removal of the brain, the superior tempero-sphenoidal and the superior marginal convolutions of the right side were somewhat softer than usual. About the middle of the superior tempero-sphenoidal convolution and 15 mm. below the trephine hole through the skull there was an area of fluctuation just beneath the surface. On handling the brain it ruptured at this point with the escape of a considerable amount of dark, grayish green, thick pus with a foul putrid odor like that of butyric acid fermentation. On incision in this region there was an abscess the size of a walnut, surrounded by several smaller abscesses varying from 3 to 15 or 20 mm. in diameter. They all had soft necrotic walls and were evidently recent. The brain tissue for some distance, 4 cm. in some places, on all sides of the main abscess was soft, edematous, and of a peculiar gelatinous consistence and appearance. The abscesses with the area of softening extended antero-posteriorly from the ascending frontal to the angular convolution and internally nearly to the right lateral ventricle. The pia-arachnoid over the abscess was markedly congested and cloudy in appearance. On section of the brain no other abscesses were to be found. The structures at the base and all the vessels appeared normal. The sinuses were free from thrombi and the middle ears and mastoid cells were normal.

The spinal cord was not removed.

Chest. The muscles were well developed; the costal cartilages were calcified. Both lungs were bound to the chest-wall by dense fibrous adhesions; both pleural cavities were almost completely obliterated. Both lungs were voluminous and on section markedly congested and edematous. No areas of consolidation were to be found. The bronchi contained frothy, blood-stained serum. The bronchial glands were pigmented, but otherwise were normal. The pulmonary vessels were normal. The trachea, tongue, and thyroid were negative. The heart, liver, kidneys, spleen, stomach, intestines, and other organs showed nothing of present interest and their description is therefore omitted.

The thick, creamy, grayish green appearance and the butyric acid odor of the pus of the brain abscess reminded me at once of pus with similar characteristics obtained some years ago from two cases of empyema from which an organism apparently belonging to the ray fungus group was obtained, so cover-slips were examined immediately during the progress of the autopsy. The pus was rich in polymorphonuclear neutrophilic leucocytes and showed a few scattered bacillus and thread-like forms. In several preparations stained by Gram's method larger and smaller clumps of branching threads were found. A few leucocytes containing bacillus and long thread-like forms were seen in some preparations. The threads varied very much in length and to some degree in thickness. The bacillus and thread-like forms varied from three to thirty or forty microns in length. As a rule they stained deeply and uniformly with fuchsin and gentian violet in either aqueous or aniline solution, but in many, especially the shorter forms, *i.e.*, those of four to eight microns in length, showed larger or smaller unstained areas which usually involved the whole thickness of the organism. In some of these forms with unstained areas a thin cell membrane was discernible. The organisms varied from 0.3 to 0.5 in thickness, the former being the average. Some forms showed irregular slight swellings, which were usually situated at one or the other end. No typical knob or club-like forms could, however, be found. The ends were usually square, were often rounded, especially in the shorter forms. Many of the longer forms were wavy in outline. Some of the shorter forms were bent, forming almost comma shapes. Some threads and rods showed transverse division into short bacillus and even coccus forms, and thus chains of bacilli and cocci were not uncommon. The latter forms were always larger than streptococcus pyogenes. The mass of intertwining branching threads showed no special spore-bearing structures; careful search failed to demonstrate the presence of clubs. The forms were non-septate, and the branching was true, the protoplasm of the main stem always being directly traceable into the branches. In the larger clumps the growth was always radiating, and the mycelium formed a distinct felt work. No capsules could be made out.

Numerous cultures, both aerobic and anaerobic on slants and on Petri plates were made on various media, including plain glycerine and glucose agar and potato with glucose bouillon and chest serum, inoculated from the pus of the brain abscess. Care was taken to use a large amount of pus for sowing the cultures. In many of the cultures streptococci, which reacted in every way to the tests for streptococcus pyogenes, grew. In none of the cultures were there forms suggestive of those found on the coverslips. Following the plan of Norris and Larkin, attempts were made to grow the organism on the organs of newly killed rabbits, but always without success. Aerobic plate cultures from the lungs gave streptococcus pyogenes and staphylococcus pyogenes aureus (a few), from the liver and kidneys streptococcus pyogenes.

A rabbit and a guinea-pig were inoculated at once introperitoneally.

The guinea-pig remained well and was killed six weeks later and showed no lesions.

The rabbit died twelve days after inoculation, and showed a wide-spread purulent peritonitis, the peritoneal cavity being distended with a large amount of thick, white, sticky pus of the consistency of thick cream, with a well marked butyric acid odor. The surfaces of the liver and spleen, as well as those of the intestines, were covered with similar material. Coverslips showed no branching forms, but numbers of long and short threads, bacillus and coccus forms, with leucocytes. Cultures from the peritoneum showed a few colonies of streptococcus pyogenes, but no other organisms.

Pus from the peritoneal cavity was inoculated into the pleural cavity of a rabbit (rabbit II.), which died in three days with extensive purulent pleuritis and pericarditis, and pus from this inoculated into a third rabbit (rabbit III.) caused its death in two days with identical lesions.

Other rabbits were inoculated intravenously with pus from the peritoneal cavity of rabbit II., died within a few minutes with extensive thrombosis of the right ventricle, and one inoculated into the trachea died during the night with purulent pleuritis. (Rabbit IV.)

Histological examination and sections from various portions of the brain showed as follows:

Brain abscess: A number of sections cut in both celloidin and paraffin and stained in hematoxylin and eosin, toloidin blue and eosin, aniline oil gentian violet, followed by aniline oil and xylol, Weigert's gentian violet method, aniline oil fuchsin followed or not with iodine, decolorized with aniline oil, and Mallory's stain for clubs; Weigert's stain followed by aniline fuchsin, and the Ziel stain for tubercle bacilli.

The abscess was made up of a thick mass of cells, most of which were polymorphonuclear neutrophilic leucocytes, among which there were both large and small mononuclear cells. About the margins of the abscess the tissue was infiltrated with the same varieties of cells, and often contained red blood cells. Many of the cells were necrotic; nuclear fragments were numerous. The brain tissue for a considerable distance from the abscess proper was edematous, and contained collections of leucocytes. The blood vessels were markedly dilated, and in many places there was marked perivascular cellular infiltration, with proliferation of the endothelium of the perivascular lymphatics. No micro-organisms were found in the blood or lymph vessels. The only micro-organisms to be found were clumps of long threads always lying near the centre of small abscesses or about the margin of a large abscess. These organisms were sometimes seen singly or in small clumps, but nearly always formed larger or smaller colonies from twenty to one hundred microns in diameter. These colonies were composed of branching nonseptate threads, which formed a meshwork and radiated from the centre of the colony. These colonies when cut through the centre were nearly round in outline and had often rather regular but somewhat serrated margins. In sections stained with hematoxylin or methylene blue the colonies are finely granular and stain homogeneously with the blue stain. No organisms were made out even at the periphery.

In very thin sections stained with eosin followed by Weigert's fibrin stain, very beautiful pictures were given. Here the colonies appeared composed of a finely granular pink ground, traversed by radiating, intertwining, branching dark blue threads, which projected from the margin of the colony in an irregular wavy line. The finely granular structureless pink staining mass appeared to support the mycelium of branching threads. The threads were usually more thickly placed at the border of the colonies, and while on first examination the outlines of the colonies were uniform, closer study showed that many threads projected for some distance from the colonies between the surrounding leucocytes. Hence the relation between the colonies and the surrounding leucocytes was an intimate one. No leucocytes were ever, however, found penetrating the colonies, although they approached individual threads. These projecting threads never formed clubs, and showed no special tendency to break up into short bacillus and coccus forms. Near the colonies both long and short free threads were to be found. Reverting to the threads in the colonies, they stained well with aniline gentian violet and both aniline and carbol fuchsin, and retained the stain tenaciously whether treated or not with Lugol's solution, or exposured to aniline oil or alcohol.

The threads were of about the same size as those described on coverslips made from the fresh pus. They often showed unstained spaces and irregular lateral swellings or bulging of the cell membrane. Many were wavy and tortuous in outline. The threads took the stain very differently — some deeply and others very faintly. No clubs were found. The threads were not septate. No spore-containing bodies were seen. Here and there among the leucocytes were larger and smaller masses of threads, some of which were evidently parts of colonies, while others represented detached masses. The threads readily yielded the fuchsin and gentian violet stains on treatment with acids in either weak aqueous or alcoholic solution, and therefore do not belong to the acid fast fungi.

Sections, including the pia-arachnoid, over the abscess and near the seat of operation, showed congestion of the blood vessels and leucocytic infiltration. No threads were found here. Histological examination of the other organs showed no acute or chronic lesions in any way suggestive of reaction to this fungus.

Animal experiments and sections of the peritoneum over the abdominal wall, liver, and intestines of rabbit I. showed swelling, desquamation, and necrosis of the endothelium in places, with an exudate of leucocytes. The leucocytic accumulation varied markedly in extent in different places. There was a notable absence of fibrin. Most of the leucocytes were of the polymorphonuclear neutrophilic type. Among the leucocytes there were in many places great numbers of bacillus and streptococcus forms. In some places long threads were seen, but no branching forms could be found. The threads were never in the form of colonies, as in the brain abscess, but were met with singly or in scattered clumps. They were of the same size, general appearance, and had the same staining characters as those described in the brain abscess. The same applies for the bacillus and coccus forms. Many of the former were curved or bent, and the latter were on the whole larger than streptococcus pyogenes. No nodular formations were present in the peritoneum or in the organs. Sections of the lungs and pleura and pericardium of rabbits II. and III. showed wide-spread purulent exudation, without recognizable fibrin, or the formation of definite nodules. The micro-organisms were even more numerous in these exudations than that of rabbit I. They had the same characters, with the exception that here clumps of definite branching forms could be found. There were no well-marked colonies, but somewhat elongated tissues of interlacing threads, some of which showed true branching. The shorter non-branching bacillus and coccus forms were, however, much more numerous. In both animals, as in the brain abscess, there was a certain amount of phagocytosis on the part of the polymorphonuclear leucocytes, some of which contained numbers of short threads or forms.

The parenchyma of the lungs of rabbit II. was not appreciably affected, and no organisms were found in other organs. The organisms had the same staining reaction noted for those in the brain abscess.*

Including Almquist's rather doubtful case, there are five known cases of primary cerebral actinomycosis, three of which have been reported as instances of "streptothrix" infections. In all except Bollinger's case, the only one to show the changes of "typical actinomycosis," the course of the disease was acute. In this case the diagnosis was brain tumor. In one case the diagnosis was tuberculous meningitis, in another, cerebro-spinal meningitis, and in one cerebral hemorrhage. Four cases were males and one female; all were adults.

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* My thanks are due to Drs. Cushing and Allen for the use of the clinical history of the case.

The organisms were cultivated in two cases (Almquist and Ferré et Fouquet), and not classified as typical actinomyces, but as "streptothrices."

Pathogenesis for animals was tried in four cases, and was positive in only one (mine).

II. SECONDARY ACTINOMYCOSIS OF THE CENTRAL NERVOUS SYSTEM.

The earliest reported case of secondary cerebral actinomycosis is that of Ponfick,[5] described in his work on actinomycosis in 1882, and quoted by O. Bollinger. A woman who had actinomycosis of the neck and prevertebral tissue developed actinomycotic masses of the left cerebral hemisphere, as well as of the pericardium, lungs, and spleen. In a second of Ponfick's cases, an actinomycotic process extended directly through the skull and invaded the pia and the right temporal and frontal lobes.

Kohler,[6] in 1884, reported the case of a man with a number of fluctuating nodules on the surface of the body which were mistaken for glanders. At autopsy there were found ulceration of the upper jaw and sternum, and abscesses of the brain, neck, tongue, spleen, liver, and kidneys, and colon. In the tenacious green pus of the various abscesses there were small greenish masses, which on microscopical examination proved to be colonies of a ray fungus. No mention is made of cultures or animal inoculations.

O. Israel,[7] in 1884, described the case of a laborer thirty-one years old, who entered the hospital with a number of ulcers and abscesses on the surface of the body. At autopsy there were found ulcers of the skin of the chest, pericardial adhesions, abscesses of the heart, spleen, liver, kidney, and intestine, and brain. Numbers of actinomyces were found in the pus from the skin lesions.

Naunyn,[8] in 1888, reported the case of a girl of sixteen years who died with the symptoms of chorea. At autopsy there were small excrescences on the mitral valve and reddish-brown masses on the cerebral pia-mater. In both lesions a branching thread-like organism was found. Cultures and animal experiments were not mentioned and were probably not made. It is interesting to recall that Zopf, to whom specimens were submitted, classified the organism as a species between Cladothrix and Leptothrix.

Keller,[9] in 1890, reported the case of a female forty years old who had pleurisy in 1885, and who in 1886 developed an abscess over the sixth and seventh costal cartilages. Actinomyces were found in the pus of these abscesses. Two years later she was taken ill with convulsions, paralysis of the left side, and coma. At operation, and later at autopsy, chronic meningitis and a small abscess at the right side of the brain were found.

In 1891 Eppinger[10] described, under the title "Ueber eine neue pathogene Cladothrix und eine durch sie hervorgerufene Pseudotuberculosis (Cladothrichica)," a case of brain abscess and meningitis associated with changes in the bronchial and supra-clavicular glands. The patient was a glass-worker, fifty-two years old, who developed what was apparently joint rheumatism. Eight days later he was unable to work. Later there was paralysis of the left side, tenderness of the right side of the head, delirium, and coma. Death occurred after an illness of twelve days. At the autopsy the skull was healthy. There was well-marked cortical, basal, and spinal meningitis, the exudate at the base of the brain being thick and sticky and of a yellow color. The brain tissue was congested and soft, the lateral ventricles were distended with yellowish pus. Communicating with the right lateral ventricle through an opening in the optic thalmus there was a large abscess of the right hemisphere extending from the middle of the frontal to the occipital lobe. The abscess was filled with pasty pus; its wall was thick and rather firm. There were firm nodules in the lungs and pleura (pseudo-tuberculosis). The bronchial and mediastinal lymph glands contained firm grayish-white nodules. The right supra-clavicular glands were very much enlarged and soft.

In coverslips, and in cultures from the brain abscess, the meningeal exudate, and the bronchial and supra-clavicular lymph glands, Eppinger obtained a branching, thread-like organism growing in star-shaped colonies which produced pseudo-tuberculosis in both guinea-pigs and in rabbits. This organism, which grew readily on ordinary culture media, has been classed by Kruse, Lehmann, and Neumann and others as Streptothrix Eppingeri. MacCullum, who has recently isolated the same organism from a case of peritonitis, correctly classes it under the actinomyces, as Actinomyces asteroides.

Sabrazés et Riviere[11] (1894) described the case of a man thirty-one years old, an alcoholic, who was said to have been ill for three weeks before admission to the hospital. Ten days before admission he showed marked mental disturbance. The day of his admission he was aphasic, had great pain in the head, vomiting, and obstinate constipation. He soon became comatose and died.

Diagnosis, tuberculous meningitis.

At the autopsy the meninges were found normal. The left cerebral hemisphere was markedly edematous, soft, and almost diffluent. There was an abscess the size of a small orange in front of the centrum ovale in the prefrontal region and involving the first and second frontal convolutions. The apices of both lungs were consolidated and one contained a small abscess. The right kidney was the seat of a large infarct. In the green pus of the cerebral abscess there were long branching filaments, some showing unstained areas. Chains of irregular micrococcus-like forms were also seen. The same appearances were met with in the renal infarct, where coccus and rod forms as well as masses or colonies of interlacing filaments were also plentiful. The colonies were circular in shape and had a radiated appearance. The ends of the filaments were regular, and showed neither swelling nor diminution in size. Sections of the brain abscess showed edema and disintegration of the brain tissue, with inflammation; but no organisms were found. Sections of the renal infarct showed them, however. No filaments were found in the lesions of the lungs. The organism failed to grow aerobically, but an anaerobic gelatine culture gave, after five days, a single colony of branching, filamentous organism like that found in the brain and kidney. Transplantation was unsuccessful. A guinea-pig inoculated with the pus from the brain died in two days of septicemia due to a micrococcus.

As no organisms were found in the pulmonary lesions, the portal of entry is obscure and the primary seat of the affection is uncertain. It is probable, however, that more careful investigation would have discovered the fungus in the lungs. The bronchial glands were not mentioned.

The authors regard their organisms and that of Eppinger as different from that of actinomycosis.

Dolore,[12] in 1896, recorded the case of cervical and facial actinomycosis with loss of consciousness, delirium, convulsions, and episthotonus. At autopsy there was found marked cerebro-spinal pachymeningitis, in the exudate of which there were yellow granules, which on microscopical examination proved to be actinomyces.

In the same year C. F. Martin[13] reported from Chiari's laboratory two cases of secondary actinomycotic abscesses of the brain. In the first, a man thirty-eight years old, with actinomycosis of the lungs, sternum, and ribs, there was a metastatic abscess of the left occipital lobe. Actinomyces were found in the pulmonary lesions, and in the pus of the cerebral abscess. In the second, a boy of sixteen years, there was actinomycosis of the right lung and chest wall, with metastatic abscesses of the right cerebral hemisphere, the right lung, and the kidneys. The secondary abscesses were acute and contained numbers of actinomyces, clubbed forms being rare. Yellow granules were visible in the pus of both the skin and the cerebral abscesses. No mention is made of cultures or animal experiments.

In 1900 Chiari,[14] under the title of "Ueber Myelitis suppurativa bei Bronchiektasie," recorded two cases of meningitis and abscesses of the central nervous system containing branching threads which he regards as identical with, or closely related to, actinomycosis. Both occurred in males with bronchiectasis. Sections of the bronchiectatic cavities which were made in one of the cases showed the same organisms found in the lesions of the nervous system; hence Chiari regards the latter as secondary in both cases.

In the first, a man forty-three years old, there was a large abscess of the cerebellum, multiple abscesses of the cord, with cerebro-spinal meningitis. In addition to Pneumococcus, bundles of branching threads without peripheral clubs were found in these lesions. There was no growth in cultures after two days, and no animal inoculations were made. The masses of threads were thin, showed true branching and took the various stains, including Gram's. Club formation was not observed. Chiari states that the organism of this case had the same microscopical appearances of those found in Martin's above described cases of secondary cerebral actinomycosis, in which the " drusen " of the cerebral abscesses lacked peripheral clubs, though the latter were present in the lesions in the other organs.

Chiari's second case was a man thirty-seven years of age who had chronic purulent bronchitis, with saccular bronchiectasis of the right lower lobe, purulent cerebro-spinal meningitis, with an abscess of the left frontal lobe. The brain abscess and sections of the wall of the bronchiectatic cavities showed numerous bundles of branching threads similar to those found in the first case, with the exception that some threads showed peripheral clubs.

No cultures or animal inoculations were made.

III. CLASSIFICATION.

It is quite evident that the micro-organism found in my case does not belong to the lower bacteria on the one hand, and lacks some of the characters usually regarded as distinctive of the typical actinomyces on the other. We have to deal with an organism which forms a mycelium of elongated branching, intertwining, or interwoven threads, which break into beaded segments forming chains of coccus and rod-like forms, but which apparently does not form clubs or club-shaped hyphæ. It caused in rabbits diffuse suppuration in the subcutaneous tissues and diffuse peritonitis without nodules and without typical mycelia, only long and short threads which sometimes branched, and coccus and bacillus forms being present in the exudates. Failure of the organism to grow in artificial media and on the organs of freshly-killed rabbits interferes seriously with its comparison with somewhat similar organisms described as actinomyces, streptothrices, and psuedo-actinomyces. The terms "cladothrix" and "leptothrix" clearly do not apply here, and the term "streptothrix," though used by Eppinger,[10] Ferré et Foquet,[8] Sobrases et Rivière,[12] Flexner,[15] Norris and Larkin,[16] Musser, Pierce, and Gwynn,[4] and others for organisms more or less closely corresponding to mine is a misnomer, as Lachner-Sandoval[17] and Hektoen[18] have clearly pointed out.

Kruse[19] and Lubarsch[20] are largely responsible for the application of the term "streptothrix" to the group. Lubarsch would include under the group name of streptothrix, placed midway between hyphomycetes and schizomycetes, all ray fungi, as well as those organisms usually classed as bacteria, which occasionally produce true branches in cultures and ray forms in the animal organism, regarding the various members of the group as transition stages. As a distinct family of the streptothrix group, Lubarsch would separate those organisms which occur in ray form in the tissues, calling them ray fungi.

Hektoen[18] strenuously objects to the bringing together in a closed circle the most typical member of the group actinomyces albus, with the most atypical or imperfect, as the tuberculomyces, for instance, simply on the strength of what many in opposition to Lubarsch and others regard as a pathological condition, namely, club formation.

Hektoen further points out the illogical and untenable position of those who use in this connection the term "streptothrix," a term used by Corda in 1839 for an entirely different family of hypomycetes. Hektoen, therefore, following the lead of Gasperini, Berestenew, and Lachner-Sandoval, insists, according to the principle of priority, upon the use of the term "actinomyces" for this group. MacCallum[21] and Abbott and Gildersleeve[22] have recently emphasized this.

Lachner-Sandoval[20] recognizes twenty-nine species of ray fungi, and as some of these forms have not been cultivated on artificial media the group may be smaller, owing to unknown duplicates. Many new species must be added if the term is to include all the branching, mycelium and club forming organisms now classed as bacteria (the acid resisting bacilli, B. diphtheriæ, etc., see Abbott and Gildersleeve[22]).

Berestenew[23] concludes that the ray fungus disease, caused by parasites of genus actinomyces, to which various names such as streptothrix, oospora, nocardia, etc., have been given, may be divided into (*a*) typical actinomycosis (such as described by Bollinger and J. Israel Wolff, Boström, and others); (*b*) atypical actinomycosis without granules in the pus and without spherical masses of the parasites in the tissues, to which belong the cases of V. Eppinger, Sabrozes et Rivière, as well as certain cases of Berestenew and others; (*c*) pseudo-actinomycosis, that is, cases with all the symptoms of typical actinomycosis, and caused by different micro-organisms, (1) those which stain by Gram and which like B. tuberculosis possess the property of forming branched threads with clubs, and (2) those caused by bacteria which do not stain by Gram's method. He describes several cases of psuedo-actinomycosis.

Silberschmidt,[24] writing in 1901, studied the organisms from seven cases of human and two of bovine actinomycosis. Of the human cases, one was unusual as involving the lungs, liver, and thigh, three were cases of infection of the lower lachrymal duct, and three were typical clinical cases of actinomycosis (jaw, mouth, and back). With the exception of two of the forms from the lachrymal duct, all showed great similarity in their cultural characters, but none corresponded morphologically with the form described by Boström as the only cause of human and bovine actinomycosis. There was great similarity to the forms described by Wolff and Israel, but there were many cultural differences.

Silberschmidt regards as similar to his the organisms from two of Berestenew's cases of so-called psuedo-actinomycosis.

He quotes Poncet and Berard's description of the points of difference between actinomycosis and pseudo-actinomycosis:

(1.) In actinomycosis the mycelium consists of hairlike, radiating threads with numerous peripheral clubs, while in pseudo-actinomycosis the mycelium is more interlaced or interwoven and not radially arranged, the branching less frequent, and the spores "lightly stained," the organism having more the appearance of leptothrix.

(2.) The actinomycosis fungus is more difficult to cultivate and may be either aerobie or anaerobic, while in pseudo-actinomycosis the growth is more luxuriant and microscopically made up mostly of forms like B. diphtheria, but with neither granules nor long threads.

After comparing the cultures from his own cases with each other and with others obtained from l'Institute Pasteur and Krâl's laboratory, Silberschmidt makes the following groups:

(1.) Growth aerobic and at room temperature, colonies on agar and blood serum are adherent to and send out numerous mycelial outgrowths into the media.

(*a.*) Liquefies gelatine, threads long, interlaced, and unbroken, includes actinomycosis hominis and bovis (Boström and others), and actinmoduræ.

(*b.*) Does not liquefy gelatine, threads broken and often appearing in short forms, actinomyces asteroides (Eppinger), actinomyces Capræ (Silberschmidt).

(2.) Colonies not adherent to media, no offshoots, gelatine not liquefied, threads mostly short with many bacillary forms, examples of the group are actinomyces farcini C, and the organism obtained in Silberschmidt's case 3.

(3.) Growth preferably anaerobic, colonies show no mycelial projections, and on solid media are usually small and sharply circumscribed. No growth on gelatine or at room temperature; the colonies are readily crushed and broken up. The organism does not survive as long as those of groups one and two on artificial media. To this group belong the cultures from six of his human cases as well as those from actinomycosis bovis.

He thinks it too early, however, to attempt a definite division of the pathogenic varieties of the genus actinomyces, and he does not regard pathogenesis as a possible means of diagnosis.

Silberschmidt concludes that:

(1.) Actinomycosis, contrary to the earlier idea, is not a specific infection caused by a single ray fungus, for a number of different micro-organisms may cause the typical disease picture.

(2.) The "drusen," which are not always to be found microscopically, may or may not have peripheral clubs, and are colonies which various micro-organisms may form in tissues.

(3.) The microscopical investigation of the "drusen," or of sections of tissue, permits the finding of the organisms, but not diagnosis of the same.

(4.) Cultures which he obtained without difficulty must be resorted to in order to distinguish the cause of the disease.

(5.) Mixed infection is not the rule in actinomycosis.

(6.) It is not at present possible to distinguish by bacteriological methods between actinomycosis and pseudo-actinomycosis.

(7.) Most of the organisms found in actinomycosis belong to the class of actinomyces, and of this class certain subdivisions are to be made.

Abbott and Gildersleeve[22] have recently pointed out that the various acid-resisting bacilli, distinct from the so-called B. tuberculosis, may form mycelial threads with or without typical actinomycotic peripheral clubs, and may cause larger and smaller inflammatory nodules in the tissues of various animals. As is well known, it has been found by Schulze,[25] Lubarsch,[20] Babes et Levadili,[27] Abbott and Gildersleeve,[22] Friedrich and Nösse,[26] and others that various members of the tubercle bacillus group and other acid-resisting bacilli, as well as streptothrix Eppingeri (Lubarsch), may form branching mycelial threads similar to actinomyces.

Abbott and Gildersleeve urge that these organisms be classed, not as bacilli, but under genus actinomyces.

Lehmann and Neumann, in the last edition of their work, classify as actinomycetes all the delicately threaded organisms free from chlorophyll, with true branching, in part very abundantly ramifying mycelium, partly with the formation of conidia. This class is further divided into the coryne-bacteria group containing B. diphtheriæ, B. mallei, and B. pseudodiphtheriæ, B. Xerosis; and Mycobacteria, a group containing B. tuberculosis and the acid-resisting, so-called, bacilli in general; and Actinomyces, a group made up of Actinomyces bovis, Actinomyces farcinicus, Actinomyces asteroides, Actinomyces Maduræ, and the various non-pathogenic actinomyces.

It is certain that our organism belongs to the branch of the actinomyces family in which the formation of branched threads without clubs is more or less constant. Its exact position, on account of its failure to grow on artificial media, must remain unknown. Its marked pathogenicity for rabbits serves to separate it from certain forms most commonly met with in the typical actinomyces of man and cattle.

SUMMARY. Actinomycosis of the central nervous system is rare, we are able to find only five, including our own case, primary and thirteen secondary cases. The disease usually affects males in the prime of life; in the primary cases the age being given as twenty-four, twenty-six, and fifty-two years, respectively, in three cases; not mentioned in two cases. In the secondary group, two were sixteen, five between thirty and forty, one forty-three, and one fifty-two years; age not mentioned in four. Two cases occurred in females. Of the primary, three were reported as instances of "streptothrix" infection and only one (Bollinger) was regarded as typical actinomycosis.

Of the thirteen surely secondary cases, eight were regarded as typical actinomycosis, two as probable actinomycosis, two as cladothrix infection, and one as streptothrix infection.

All of the primary cases except one (Bollinger) were acute, while of the secondary in at least ten the lesions of the nervous system were recent, and usually the cause of death.

The organisms were cultivated in two primary (Almquist and Ferré et Fouquet), and in two secondary cases, in one of which (Eppinger's) aerobic and in the other (Sabrozes et Riviére) anaerobic growths were obtained. Eppinger's organism was pathogenic for rabbits and guinea-pigs. In most of the cases neither cultures nor animal experiments were made, the diagnosis being made from the microscopical examination alone. No mention is made of any attempts to test the acid-resisting powers of any of the organisms found. The so-called actinomycotic granules were recognized in the pus of several cases. In one, instead of abscesses there were multiple nodular masses in the brain, and in Bollinger's case there was a firm mass the size of a hazelnut in the third ventricle and in the foramen of Monro. Primary lesions of the lungs and bronchial glands were the most common sources of the metastatic lesions of the central nervous system. In one case the actinomycotic process extended directly through the skull to the meninges and brain.

It seems established that, in the present state of our knowledge, all processes caused by micro-organisms having a mycelium of branching, interlacing, and sometimes radiating threads are to be considered as actinomycosis, and that organisms with the above characteristics, whether or not they form "drusen" and clubbed hyphæ in the tissues, are to be regarded as actinomyces.

Further knowledge is necessary before a satisfactory classification of the actinomyces and the processes caused by them is possible. To class tuberculosis under actinomycosis is of doubtful value. It is well to recall, however, that there are two broad divisions of genus actinomyces as used by Abbott and others, the acid-resisting (B. tuberculosis and the acid-resisting, so-called, bacilli) and the non acid-resisting or acid-bleaching, and that typical tuberculosis is always caused by the prominent member of the former, and typical actinomycosis by the prominent member of the latter group. It is better, with Lehmann and Neumann, to use the term "actinomycetes" for the large number of organisms of the latter group. This acid-bleaching group is apparently a large one, and our knowledge concerning it is best summarized in Silberschmidt's conclusions.

The organism of our case belongs to the branch of this group in which club formation is inconstant or absent, pathogenesis for animals is marked, and cultures on artificial media negative.

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AN EXAMPLE OF A PROPOSED ARRANGEMENT OF SPECIMENS OF A SINGLE STRUCTURE FOR MUSEUMS.[1]

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When the idea was advanced, years ago, that a museum is a collection of labels with specimens behind them, I thought it was, to say the least, very far-fetched. It seemed, moreover, a little degrading, a little beneath the dignity of a serious museum; for, as I thought, the real student should know enough to appreciate the specimens themselves. Even now, though I have fully accepted the proposition, I am not free from a certain anxiety lest information so presented should lead to superficiality, for we know

> "How index-learning turns no student pale,
> Yet holds the eel of science by the tail."

On the other hand it must be recognized that with the advance of specialization there are few men quite at home in more than a very limited number of subjects (if indeed we should not say parts of subjects), and for these it is an immense advantage to be told in few words what is taught by specimens which they are capable of understanding, but with which they are not familiar. Hence the need in museums of careful classification and of proper labeling.

Now to apply this to a collection of human anatomy. There must be first, certain more general series, showing, for instance, the structure of bone and the various types of muscles, and then a large number of special series showing the variations of each individual bone and organ and the peculiarities depending on sex and age. Then should follow specimens of the rarer anomalies. For such a collection to be valuable two points are of importance: the number of specimens must not be so great as to be confusing, and the

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labels must be large enough to be easily read at a distance and must tell the story clearly.

It is needless to say that the maker of such a collection must be sincere. It should not be made for special pleading, but to tell the truth. Were it possible, such collections should represent no theory at all; but in many cases this is impossible, for the labels must tell the story as the teacher understands it. Still, even if he tell it wrong, there are the specimens at hand to contradict him. Though they may have been brought forward to testify to one side, they are subject to cross-examination.

Many valuable and extremely rare specimens have been added of late years to the collection of normal anatomy in the Warren Museum; but they are lost to all but the very well informed, owing to the defects of the room and to the want of proper classification and labeling. There are two series which may be called relatively complete. One is that of thin sections showing the internal structure of bone. There are long series of successive sections without a break through many of the bones of the human skeleton, and there are also series showing corresponding sections in different animals. I must not forget to mention how much this collection owes to the skill of Dr. S. J. Mixter. The defect is in the labels. One to be sure accompanies each tablet, but they are too small to be easily read and not sufficiently explanatory.

I have taken great pains to make the collection of spines, which so far as I know is unequalled, easily understood by any one familiar with the rudiments of anatomy. The story is told by the following series of labels:

NUMERICAL VARIATIONS OF VERTEBRA.

CLASS I. — Normal number of presacral vertebræ.
Group A. Irregularity at junction of back and loins.
Group B. Irregularity at junction of back and neck.

CLASS II. — Tendency to increase of presacral vertebræ. Twenty-fifth vertebra is partly sacralized.

The series shows diminishing sacralization of the twenty-fifth.

CLASS III. — An extra free presacral vertebra.
Group A. The extra vertebra in back.
Group B. The extra vertebra in loins.
Group C. Two extra vertebræ.

CLASS IV. — A tendency to diminution of presacral vertebræ.
Group A. Fusion of vertebræ.
Group B. Fusion of atlas and skull.
Group C. Cervical vertebra suppressed.
Group D. Twenty-fourth vertebra partly sacralized.
This series shows increasing sacralization.

CLASS V. — One presacral vertebra wanting.
Group A. Vertebra wanting in loins.
Group B. Vertebra wanting in back.
Group C. With cervical ribs.

The specimens marked * are described in Professor Dwight's monograph on numerical variations of the spine. (Memoirs of the Boston Soc. Nat. Hist., Vol. V., 1901.)

It has long been my wish to have the range of variation within normal limits, as well as the occasional anomalies of all the distinct bones of the human skeleton, exhibited in a system of series, and for many years I have collected material. As long ago as 1887 I published in the "American Naturalist" a paper on the "Range of Variation of the Human Shoulder-Blade," and in the Shattuck Lecture of 1894 I called attention to peculiarities of other bones. To-night I present a series of shoulder-blades with their labels as an example of what it is proposed to do for the skeleton and then for other organs. It is ready to place in the museum, and again I leave the labels to tell the story. Before yielding them the floor I wish to express my indebtedness to Dr. Whitney, the Custodian of the Museum, for his interest in the plan and his valuable assistance.

The following labels accompany the collection of scapulæ:

SCAPULAR INDEX $= \frac{\text{Breadth x 100}}{\text{Length.}}$ A low index implies a relatively long scapula. Average index of whites, about 63.5. Extremes of whites, 55 and 82. The lower races have higher indices.

Extremes of Length. — 1, male, 20 cm.; index, 56.5. Both length and index near extremes. 2, female, 13 cms.; index, 73.1. The shortest white bone measured by Professor Dwight.

Average Male and Female. — 1, male bone; 2, female bone. The average bone is not typical.

Nearly Extreme Indices. — 1, 55.5; perhaps the lowest observed. 2, 76.9; very high. The race of both is unknown.

Posterior Border. — 1, straight; 2, convex; 3, concave, uncommon.

Upper Border. — 1, nearly straight; 2, descending slightly; 3, descending steeply; 4, with enlarged superior angle; 5, with truncated superior angle.

Supra-scapular Notch. — 1, absent; 2, barely indicated; 3, shallow; 4, very deep; 5, almost bridged over; 6, foramen; 7, foramen and notch; 8, double foramen. The last is perhaps unique, and the next very rare.

Different Forms of Acromion. — 1, the quadrate; 2, the trigonal; 3, the spatulate. *Vide* Macalister, Journ. of Anat. and Phys., Vol. 27.

Inclination of Acromion. — 1, approximately horizontal; 2, intermediate. Approximately vertical.

Irregular Ossification. — Adult male Italian. Separation of terminal epiphysis and presence of several smaller centers. (The other side was much the same.)

Ossification of Boracoclavicular Ligaments especially of the Conoid. (Probable traumatism.)

The Scapula during Growth.[1]

Chinese. — Male, aet. 31; scapular index, 72.4.

Inferior Angle. — 1, sharp; 2, medium; 3, rounded; 4, process for teres major. This process is much developed in some monkeys.

Terminal Epiphysis of Acromion remaining free and in this case lost. — Male, aet. 37.

The smaller labels formerly on these bones have been removed and stored with those from a great many other scapulæ. They show in most cases the race, sex, age, color, height, breadth of shoulders of the body from which they came, as well as the length, breadth, and index of the bone. These are available for the use of special students.

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[1] This label is not filled out, as better specimens are hoped for.

THE ADRENAL GLAND AND ITS ACTIVE PRINCIPLE IN THEIR RELATIONS TO CYTOLYSINS AND ANTITOXIN PRODUCTION.[1]

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(*Read before the Association of American Physicians, May, 1903.*)

I. Introduction. In the course of an investigation upon the relation of certain bacterial toxins to particular organs of the body, the details of which need not concern us at this time, it became desirable to experiment upon guinea-pigs from which the adrenal bodies had been, in part or wholly, removed, or in some other manner rendered physiologically inactive.

Our efforts to meet this requirement have led us far from the original object of the research, but at the same time have supplied information that we regard as of sufficient general interest and importance to justify its embodiment in a special communication.

As is well known, removal of the adrenal bodies from guinea-pigs by the customary surgical procedure is almost invariably fatal within a few hours. Our own experience in this field was in no wise different from that of other investigators.

Destruction of the glands by the direct injection of corrosive substances, such, for instance, as strong iron chloride solutions, was in our hands likewise fatal within from six to ten hours, so that both of the foregoing procedures were abandoned as unpromising after a few trials.

At the time these experiments were begun,[2] the state of our knowledge upon the reactions in the animal body resulting from the introduction of alien cells, fluids, and tissues led us to hope that the treatment of animals with the fresh adrenal cells of the guinea-pigs would ultimately result in

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[1] Received for publication April 4, 1903.

[2] Spring of 1902.

the appearance in the blood of the treated animal of substances having a specific, probably specifically destructive, relation to the guinea-pig's adrenals in situ; and that by the use of such specific cytolytic sera upon guinea-pigs the partial or complete elimination of their adrenal glands might be accomplished; and this, too, in so gradual a manner as not to endanger the life of the animal to the same degree as is usual with the surgical procedures. We were encouraged in this hope not only by the ingenious doctrine applying to the artificial induction of cytolysins in general, but more particularly by the investigations of Bigart and Bernard[1] that had a direct bearing upon the problem before us. These investigators state that after the intraperitoneal injection of ducks with guinea-pig's adrenals the sera of the ducks, when injected into the peritoneal cavity of the guinea-pig, manifest a specific affinity for the adrenal glands. This is evidenced by a swelling of the adrenals without alteration of shape, and by more or less complete discoloration and gelatiniform softening of the medullary portion of the glands. The authors state that the microscopic alterations of the gland are profound, but reserved the detailed description for a subsequent publication.

As we have not encountered their later communication, it is scarcely possible from the foregoing description to form an adequate conception of either the local or general conditions produced in the animals upon which they operated.

II. METHOD OF IMMUNIZATION. — In the studies to be here recorded an effort was made to secure a specific cytolytic serum for the adrenals of the guinea-pig by the repeated intraperitoneal injection of rabbits with freshly prepared infusions of guinea-pig's adrenals. The glands were removed from normal animals, of as large size as could be secured, were carefully freed from all adherent blood by washing in 0.85 per cent sodium chloride solution, were then rubbed up in a porcelain mortar with the same solution, and, after straining through cheese cloth, were injected directly into the peritoneal cavity of the rabbit by means of hypodermic syringe, strict aseptic precautions having been observed throughout. No deaths resulted from infection after such injections, and we were surprised to find that the effects, in general, upon the rabbit were as a rule inconsiderable, there being neither evident discomfort, disturbance of circulation or respiration, nor either elevation or depression of rectal temperature. The number of adrenals injected at one time as well as the interval between the injections varied. The maximum number of glands used for a single injection was six, and the intervals between the injections were from three to ten days. In all, five rabbits were employed.

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[1] Compt. rend. heb. d. l. Soc. d. Biol., 1901, p. 161.

Rabbit A received 23 guinea-pig adrenals between Sept. 8 and Dec. 5, 1902; Rabbit B, 31 adrenals between Sept. 8, 1902, and Jan. 23, 1903; Rabbit C died after the second injection of two adrenals with nothing to account for death; Rabbit D received 32 adrenal glands between Dec. 16, 1902, and Feb. 26, 1903, and Rabbit E received 38 adrenals between Dec. 26, 1902, and March 19, 1903.

In all the rabbits treated in this manner there was a progressive loss of weight, reaching ultimately between two hundred and fifty and three hundred grams. The weight of one of the animals (Rabbit E) fell from 1,960 grams at the beginning of the immunization (on Dec. 26, 1902) to 1,200 grams on March 19, 1903. This fact renders it somewhat doubtful if we could have kept our animals alive had larger numbers of the glands been employed for the injections. Two of the rabbits were bled and the action of their serum tested after they had received seventeen adrenals each, one after fourteen and a fourth after thirty-eight. One of the five original rabbits (Rabbit C) died so early in the course of immunization that it was discarded.

The method of bleeding employed is that of Latapie,[1] which for neatness, despatch, and general satisfaction is so far superior to any other means tried by us that we have abandoned all other procedures for it.

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[1] Annales de l'Institut Pasteur, 1900, Tome XIV., p. 106.

III. EFFECT OF THE IMMUNE-SERUM ON GUINEA-PIGS.—The most striking effect upon guinea-pigs of intraperitoneal injection of serum obtained from rabbits after this plan of treatment is seen within a few minutes of injection. In five minutes, more or less, after a dose of from one-half to one cubic centimeter, according to the activity of the serum, the pig may settle upon its belly or its side, with legs alternately flexed and extended. It may be unable to stand, and if placed upon its feet falls almost at once into the prone posture. Occasionally the immediate effects are less marked, though some degree of depression is invariably observed. Its rectal temperature is often quickly reduced, reaching, after another fifteen or twenty minutes, one and a half and even two degrees below normal. At times these acute symptoms disappear within one or two hours and the animal may go on to recovery; again the conditions may become progressively worse and death supervene in a few hours. Almost immediately following the injection there is profuse urination, and in from seven to ten hours the urine may be blood stained, though this is not invariably the case. Movements of the bowels commonly follow the injection; at times they are more moist than is common to the guinea-pig; we have not seen them to be blood stained.

In animals that succumb within twelve to twenty-four hours, there is often to be observed varying degrees of subcutaneous edema; constantly an enlargement and often congestion of the subcutaneous lymphatics; a yellowish discoloration of the subcutaneous fat; at times blood-stained serum in the serous cavities; a purplish tinge to the muscles and membranes; enlargement and injection of the retroperitoneal lymph glands; and a swollen, soft and almost black condition of the spleen, this organ being frequently very much enlarged.

In animals that have died within a few hours after the injection, the liver is often congested. If death be postponed, the organ may be marked by irregular bright red areas, paler than the surrounding parts of the organ; these are seen not only upon the surface, but may extend well into the substance of the liver; where death is still more delayed, the liver may be in general of a distinct yellow or ochre color. Both the gall bladder and the urinary bladder are frequently distended with highly colored contents, the latter containing usually more or less of blood. The cortical portions of the kidneys, especially of animals that die, roughly, in about three days after the injection, are often (not always) of a dark chocolate color, while the pyramids are stained of a dirty slate color. The lungs may be normal, may be uniformly congested, or may present irregular tiny areas of consolidation, having the appearance of thrombotic areas.

The blood is frequently conspicuous for its peculiar "thin" appearance, being often so poor in normal coloring matter as to be almost transparent when viewed by transmitted light through such diaphanous tissues as the omentum or mesentery. The clots in the heart are paler than usual and have more of a transparent ruby color than that of the dark opaque masses peculiar to normal blood seen after death in this situation. Tested by the Talquist process, the shade given for such blood is often (not always) scarcely more than that indicated on the scale by a hemoglobin content of ten per cent.

The adrenals may be normal, congested, or darkened in color, according to the time that has elapsed between the injection and the death of the animal. They are not enlarged, and on section show nothing that suggests to the naked eye a disturbance of the normal condition, relations, or proportions of the zones composing them.

In brief the entire picture suggests a rapidly acting, blood-destroying process, an impression that is borne out by microscopic study of the organs.

EXHIBIT I. — EXPERIMENT OF JAN. 4, 1903.

Intraperitoneal injection of guinea-pigs with "adrenal serum" from Rabbit B.

Pig.	Weight.	Dose of untreated serum.	Dose of serum heated to 55–56° C. for 30 min.	Dose of heated serum and fresh infusion of adrenals.[1]	Rectal temp. within 1 hour after injection.	Results.
14	490	1.5 cc.	....	....	34.6° C.	Dead in 3½ days. Blood thin; liver pale yellow; fat yellow; kidneys dark chocolate color; irregular areas of consolidation in lungs; adrenals not certainly affected; spleen a little swollen; hemoglobinuria.
15	430	1.5 cc.	....	....	34.0° C.	Dead in 5 hours. Vessels of mesentery and omentum injected; kidneys and adrenals very much congested; areas of consolidation in lungs; serous tissues and muscles of a diffuse purple color.
16	380	....	2 cc.	....	35.8° C.	Dead in 3½ days. Counterpart of Pig 14, except that the spleen was more involved.
17	370	....	2 cc.	....	33.2° C.	Dead in something less than 3 days. Blood thin; liver ochre color; fat yellow; bile dark brown; hemoglobinuria; kidneys dark chocolate color; spleen only moderately swollen and darkened; adrenals not perceptibly altered.
20	440	....	....	4 cc.	38.3° C.	Dead in 92 hours. Yellow fat; pale yellow liver; chocolate-colored kidneys; consolidated areas in lungs; blood in urine; blood thin and watery; adrenals not certainly affected, did not differ from those of foregoing animals.

EXHIBIT I. — *Concluded.*

Pig.	Weight.	Dose of untreated serum.	Dose of serum heated to 55-56° C. for 30 min.	Dose of heated serum and fresh infusion of adrenals.[1]	Rectal temp. within 1 hour after injection.	Results.
21	460	....	....	4 cc.	40.0° C.	Dead in less than 17 hours. Consolidated areas in lungs; adrenals a little darker in color than usual; mesentery and omentum injected; liver and spleen normal; tissues in general of a purplish color; intestines contained a few cc. of dark brown mucoid fluid; kidneys congested; small quantity of blood-stained serum in both pleural cavities.

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[1] For this test two freshly removed guinea-pig adrenals were rubbed up in the mortar with 5 cc. of sterile 0.85 NaCl sol. This was mixed in equal parts, after straining through gauze, with the serum and 4 cc. of the mixture used for injection after it had stood at 38° C. for 30 minutes. By this plan the actual dose of serum for each animal was *2. cc.*, as in animals 16 and 17.

In marked cases microscopic examination of the blood reveals the presence of erythrocytes in all stages of disintegration and distortion; many of them appear fused together into soft tenacious masses that, by the pressure of the cover-slip, are pressed out into long, adherent, "sticky" bands or irregularly outlined masses. While many normal erythocytes are to be seen, there are also many that are irregular in size and form, and many that are much paler than usual.

Microscopic examination of the liver shows the capillaries of the irregular, bright red areas, mentioned above, to be conspicuously filled with blood and to be surrounded by liver cells in varying stages of degeneration. Little or nothing of nuclear fragmentation is to be observed, and the change noted gives the impression of being due more to circulatory disturbance than to the action of specific intoxicants upon the tissues. It is possible that these lesions may result from the occurrence of agglutinative thrombi, though on this point we hesitate to speak with certainty. In the advanced cases where the liver presents the uniform yellow color mentioned it is found on microscopic examination to be in a condition of diffuse fatty degeneration with occasionally here and there islands having somewhat the appearance of hyaline change.

The spleen is usually markedly congested, and is often conspicuous for the presence of large phagocytic cells, many of which are filled almost to bursting with erythrocytes in various stages of deterioration. Often the sections are marked by the presence of large round or oval bronze colored masses evidently representing blood coloring matter. No definite crystal forms were observed. In two or three cases the cells of the lymph nodes of the spleen gave evidence of destructive changes, many of the nuclei being distorted and fragmented.

The kidneys frequently present a most striking picture. They are markedly congested. There is often to be observed ruptures of the glomerular capillaries with escape of blood into the capsule; and the epithelium of both the convoluted and the looped tubules is conspicuously marked by the presence of closely packed tiny round or oval, brownish granules, evidently the débris of destroyed erythrocytes.

On section the adrenals are usually found congested, though this varies. Rarely there are tiny points of hemorrhage. But definite necrosis or degeneration has not been detected in any of our animals. Early in the work we were encouraged by discovering evidence of cell multiplication in these organs, but subsequent study, not only of additional cases in this group, but of the adrenals from normal guinea-pigs as well as from those dead of other causes, lead us to believe that nuclear division may be commonly observed in the cells of this organ in health as well as in disease. It may be of interest to note that whenever karyokinesis was observed it was most pronounced in "zona fasciculata" near its junction with "zona glomerulosa."

By microscopic as well as macroscopic examination we have found no regular difference, save for the varying degrees of congestion and slight alterations in color, between the adrenals of these and those from normal animals or from animals dead after other modes of treatment.

The foregoing composite picture drawn from a number of animals treated with serum from rabbits that had received injections of guinea-pigs' adrenals so forcibly suggests blood destruction as the underlying cause of death and the conditions found, that a series of control experiments, designed to acquaint us with the changes resulting from the injection of actively hemolytic sera, were instituted. For this purpose guinea-pigs were injected with sera as follows: in the one group with sera from rabbits rendered highly immune from guinea-pigs' erythrocytes; in the other with the serum of normal rabbits, which, as is known, possesses more or less hemolyzing power for guinea-pigs' erythrocytes, at least in vitro.

The results of intraperitoneal injection of guinea-pigs with normal rabbit serum may be dismissed in a few words. While rabbit serum is hemolytic (in varying degrees) for guinea-pigs' erythrocytes in the test tube, it is not necessarily destructive to life when injected into the living animal, and may often be used in large doses with no effect whatever, though this is not invariably the case. For instance, two of our animals (Pigs 95 and 96 — the one weighing 260, the other 270 grams) received intraperitoneal injections of such relatively large doses as five and one-half cubic centimeters each of normal rabbit serum without more than temporary inconvenience that passed off in about an hour, and from which the animals ultimately recovered; while others (Pigs 3 and 4, weighing respectively 210 and 190 grams) succumbed to the intraperitoneal injection of three and three and one-half cubic centimeters, respectively, of normal rabbit serum; both dying in seven days after the injection, neither presenting positive evidence of a destructive action of the serum upon the blood or organs, and the peritoneal cavity of both being sterile by culture tests. From the variations in the hemolytic activity of serum from different normal rabbits, as exhibited upon guinea-pigs' erythrocytes in vitro, it is reasonable to expect variations in other properties as well, and the foregoing discrepancy may be, in part at least, explained in this way. The hemolytic activity of one of the sera mentioned above (that used in the large doses) was determined in vitro, and was found to be conspicuously low as compared with the average for rabbit serum upon the red corpuscles of the guinea-pig.

With the serum from rabbits rendered highly immune from guinea-pigs' erythrocytes, on the other hand, the results were quite different. These sera when highly hemolytic (*i.e.*, when from highly immunized animals) are almost invariably quickly fatal to guinea-pigs by intraperitoneal injection, even in comparatively small doses.

Thus, for instance, the following results of intraperitoneal injection of guinea-pigs with specific hemolytic serum were observed. The serum used was from a rabbit that had received by intraperitoneal injection seventy-five cubic centimeters of defibrinated guinea-pigs' blood in broken doses.

Exhibit II.

Pig No.	Weight.	Dose.	Rectal temperature 15 min. after injection.	Results.
24	310	0.5 cc.	35° C.	Dead in less than 3 days. Hemoglobinuria during last day.
25	320	1.0 cc.	36.9° C.	Dead in 24 hours. Hemoglobinuria.
26	340	1.5 cc.	35.8° C.	Dead in less than 2 days. Hemoglobinuria.
27	390	2.0 cc.	35.1° C.	Dead in less than 2 days. Hemoglobinuria.
Control	....	....	38° C.	

The symptoms exhibited by these animals immediately after the injection are identical, save in degree, to those noted in the animals that had received the adrenal serum,[1] and the conditions found post-mortem are likewise not essentially different. Beyond the fact that the lesions are more marked, there is the same involvement of lymphatics; the same irregular markings and more pronounced degenerations (according to the date of death) of the liver; the large, soft, almost black spleen; the frequent brown discoloration of the kidneys; the widespread evidence of blood destruction with hemoglobin staining of the tissues; and the same indefinite effect upon the adrenal glands that were noted in the animals dead after injection with the "adrenal serum." In animals that survive for a longer time than those cited above, some one or another of these features may be modified, but it is the rule to find recent or remote evidence of blood destruction, degeneration of the liver, alterations in the spleen, and either discoloration or congestion of the adrenal bodies. In the animals that die within forty-eight hours of the injection, the gall bladder and urinary bladder are usually distended with dark fluids, in the latter case evidently blood stained. In animals that survive for several days, these conditions may be less conspicuous or even absent. Hemoglobinuria within twenty-four hours of the injection is practically always seen, especially if an actively hemolytic serum be used. From our observations, the conditions of guinea-pigs after injection with an actively "hemolytic serum" do not differ except in being more pronounced from that following injection with "adrenal serum," and it is furthermore usually impossible to distinguish by either macroscopic or microscopic examination between the tissues from the two groups of animals.

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[1] For the sake of brevity, the expression "adrenal serum" will be used for the serum of rabbits that had received intraperitoneal injections of fresh emulsions of guinea-pigs' adrenals; and the term "hemolytic serum" for that of rabbits immunized from guinea-pigs' erythrocytes.

IV. EFFECTS PRODUCED BY MODIFIED SERUM. — When it became obvious that a conspicuous property of the "adrenal serum" is a marked degree of hemolyzing power, it seemed possible for our failure to secure evidence of specific action of this serum upon the adrenal glands in situ to have been due to the death of the animals from destruction of blood, before the specific effects of the serum upon the adrenals was made manifest.

With this in view an effort was made to separate from the adrenal serum its hemolytic receptors. To this end, adrenal serum, heated to 55–56° C. for thirty minutes to destroy complement, was mixed with an equal volume of washed guinea-pigs' erythrocytes, placed in the incubator for two hours and, in some cases, used for injection at once, in others left at ice-chest temperature over night. There was always a marked agglutination of the red corpuscles, so that their separation from the fluid portion of the mixture was a matter of no difficulty. An "adrenal serum" so treated, when injected into guinea-pigs in amounts equivalent to the fatal dose of untreated serum, was without effect, either immediate or remote.

By heating the adrenal serum (to 55–56° C.) for thirty minutes, its acute effects noted above are not necessarily modified — the receptors in the serum find, therefore, their appropriate complement in the body of the guinea-pig. By mixing together heated "adrenal serum" with a fresh infusion of guinea-pigs' adrenals in 0.85 per cent sodium chloride solution, its fatal hemolytic effects upon the guinea-pig are not necessarily eliminated, death often resulting from destruction of blood just as was seen to follow the use of the unheated serum. (See pigs 20 and 21, Exhibit I.)

As stated above, the immediate effects of the untreated serum, either the "adrenal serum" or the specific "hemolytic" serum, are marked, being manifested by extreme prostration with coincident depression of rectal temperature. By mixing such sera with either washed guinea-pigs' erythrocytes or with fresh infusions of guinea-pigs' adrenals in 0.85 per cent sodium chloride solution,[1] as outlined above, the immediate effects of the sera are, as a rule, caused to disappear, the animals showing practically no depression of temperature or irritation after the injection.

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[1] In the mixtures we have used washed guinea-pigs' erythrocytes in equal value with the serum. Where adrenal infusions were employed, two glands were usually rubbed up with eight to ten cubic centimeters of 0.85 per cent NaCl solution, and this added to the serum in equal parts. The necessary corrections for these dilutions were made in adjusting the dose of serum.

V. REACTIONS OF THE SERUM IN VITRO. — These results, though disappointing in so far as our desires were concerned, are not devoid of interest and gain in this respect, through the fact that the test tube reactions of the several sera employed were with one exception (adrenal serum from rabbit E) just as one would have anticipated. The untreated adrenal serum was actively hemolytic for guinea-pigs' erythrocytes, and actively agglutinative for fresh infusions of adrenal glands.

By heating (to 55° C.) for thirty minutes the hemolyzing action was checked, if carefully washed guinea-pigs' erythrocytes were employed, but there was always some agglutination to be seen, and the agglutinative action of the serum upon the adrenal cells was unaltered.

We endeavored to detect a lytic effect of the "adrenal serum" upon the adrenal cells in the test tube, but with little or no success. Agglutination was with one exception always observable and appeared at times within a few minutes after the mixture of serum and infusion was made.

Similar tests with the specific "hemolytic" serum of rabbits treated with guinea-pig blood gave, in the test tube, hemolysis or agglutination of washed guinea-pigs' erythrocytes, according to the manner of testing — but never the least effect upon the adrenal gland infusions.

The "adrenal serum" from one of the rabbits in the group offered a confusing exception to practically everything that has been said above. This animal (*Rabbit E*) received thirty-eight guinea-pig adrenals (between Dec. 26, 1902, and March 19, 1903). Six days after the last injection of six adrenals ground up in twelve cubic centimeters of 0.85 per cent sodium chloride solution, it was bled for the first and only time. Its untreated serum in gradually ascending doses (from 0.5 cc. to 2.5 cc. inclusive) had nothing more than a temporarily depressing effect (tolerably marked in the larger doses) upon guinea-pigs of from two hundred and thirty to two hundred and seventy grams weight. The serum, after heating (to 55°–56° C.) for thirty minutes, had no immediate effect upon guinea-pigs of two hundred to two hundred and twenty grams weight, when given in doses of one and a half and two cubic centimeters, respectively, intraperitoneally. One of these pigs died after five days with nothing to account for death. There had been no hemoglobinuria, and the autopsy revealed no evidence of blood destruction or of involvement of the viscera. When the serum was heated and left in contact with washed guinea-pigs' erythrocytes (two parts of serum to one of erythrocytes), by the customary plan for the removal of hemolytic receptors, it had no immediate effect upon four guinea-pigs of from two hundred and ten to three hundred and thirty grams in weight when administered intraperitoneally (in doses of 2.5 cc., 2.5 cc., 3.0 cc., and 4.5 cc. respectively). One of the four pigs (No. 129) died in two days after intraperitoneal injection of 2.5 cc. of the serum. There was no evidence of blood destruction, and careful inspection showed the organs to be normal.

As compared with the serum of a normal rabbit, selected at random, the hemolytic activity of this serum upon washed guinea-pigs' erythrocytes in the test tube was from four to five times as great, that is to say 0.01 cc. of the "adrenal serum" brought about the same degree of hemolysis in the standard suspension of washed guinea-pigs' erythrocytes as was caused by 0.04 to 0.05 cc. of the normal serum.

After heating, its hemolyzing action naturally disappeared, though it was still actively agglutinative for the red corpuscles of the guinea-pig.

But in neither the unheated nor the heated condition had it the slightest effect upon a fresh infusion of guinea-pigs' adrenals when mixed in the proportions of 0.05 cc. and 0.1 cc. of the serum to 2 cc. of an infusion of two glands in 15 cc. of 0.85 per cent NaCl solution.

We have no satisfactory explanation for this anomaly. The rabbit had lost weight, it is true, but was otherwise free from disease at the time it was killed, *i.e.*, immediately after it was bled. An interval of six days had elapsed between the date of the last injection of adrenal infusion and the time of bleeding. This may have been too brief. It is possible that we have here an individual exception to the general rule. This is not unknown in experiments of this character.

VI. DEFINITE RESULTS.—Concerning the condition of the adrenal bodies after the injection of these two types of sera into guinea-pigs, it is important to note that specific degenerative or necrotic lesions, reasonably referable to the action of the sera, were encountered on but two occasions, and singular to say these were among the animals that had received the "hemolytic" rather than the "adrenal serum." In one animal (pig 92), dead after an injection of "adrenal serum," that had stood in contact with a fresh infusion of guinea-pigs' adrenals by the customary method, both adrenal bodies were found markedly enlarged, and in both karyokinesis was so much in evidence throughout the cells of zona fasciculata as to leave little doubt of its relation to the treatment to which the animal had been subjected; though, as said above, nuclear division in varying degrees is so commonly observed in these glands as to render its significance in this particular case more or less doubtful. Moreover, the contact of the serum and the fresh infusion of the gland in the test-tube before the injection should, theoretically at least, have robbed the serum of those principles having specific affinities for the cells of the gland in situ.

The protocols of the pigs in which the adrenal bodies were definitely affected (pigs 89 and 94, experiment of Jan. 24th with "adrenal serum" and "hemolytic serum") are briefly as follows:

Pig 89: 250 grams in weight, received an intraperitoneal injection of 1.25 cc. of "hemolytic" serum that, after heating to 55–56° C. for thirty minutes, had stood in contact with an equal value of washed erythrocytes of the guinea-pig (two hours at 37–38° C. and over night in the ice chest). Theoretically the hemolytic receptors should have been removed. It died about ninety-six hours after the injection. Hemoglobin was not certainly present in its urine at any time, though the urine was highly colored. At autopsy the subcutaneous fat was yellowish in color; lymphatics enlarged though not hemorrhagic or congested; peritoneum normal, though the fat was of a yellowish tinge; the liver was of a uniform yellow or ochre color; gall bladder distended with dark colored bile; intestines normal; spleen dark in color and only moderately enlarged; both kidneys pale;

left adrenal of a yellowish color and there was a tiny subcapsular hemorrhage toward its posterior extremity; right adrenal ditto, except for the superficial hemorrhage; lungs normal; myocardium and its lining membrane, especially the latter, somewhat hemoglobin stained. Blood "thin" and generally "watery" in appearance. Clots in the heart less dark in color than normal. Highly colored urine in the bladder.

Culture tubes inoculated from the peritoneal cavity remained sterile.

MICROSCOPIC STUDY. — Zona fasciculata in both adrenal bodies marked by areas of irregular size and outline in which there was more or less destruction of tissue. There was conspicuous invasion of these areas by leucocytes, many of which contained eosinophilic elements, and at a number of points there was evidence of fragmentation of the nuclei of the fixed cells. The capillaries traversing these areas were conspicuously filled with blood and at points were marked by large numbers of polymorphonuclear leucocytes packed within them. Many of the eosinophilic cells observed in these sections — and they were numerous — were much smaller than usual, were not distinctly granular, as one commonly sees them, and gave the impression of being undeveloped "eosinophiles." Throughout the organs were a number of isolated areas in which the capillaries were very much engorged with blood, at times almost to the point of bursting, without there being at the same time any discernible necrosis or degeneration of the immediately surrounding tissues. The points at which necrosis was beginning were marked by a conspicuous accumulation of polymorphonuclear leucocytes.

There is no evidence of proliferation of the fixed cells of the organ, other than an occasional karyokinetic figure. Beyond congestion the medullary portion of the organ was unchanged. It is reasonable to refer these degenerated areas to the development of agglutinative thrombi in the vessels supplying them, though the treatment to which the serum had been subjected before it was used aimed to remove this principle. In a number of our tests in vitro, however, we found that not all of the agglutinative principle in a specific hemolytic serum is necessarily removed by such an arbitrary combination as serum and erythrocytes in equal volumes, these being the proportions used in this experiment.

The liver was marked by widespread fatty degeneration varying in extent in different portions of the organ. There were no circumscribed areas of necrosis and no evidence of nuclear fragmentation.

The kidneys did not present the condition of pigmentation so often seen in these organs after injection with a hemolytic serum, nor can it be certainly said that rupture of the glomerular capillaries was observed, though at a few points tiny areas suggested this accident.

Pig 94: 370 grams in weight, died in about seventy-two hours after intraperitoneal injection of 1 cc. of a "hemolytic serum" which, after heating for 30 minutes to 55°–56° C. had stood in contact with a fresh infusion of guinea-pigs' adrenals in 0.85 per cent NaCl solution. By this preliminary treatment the principles, if any there were, having affinities for the adrenal tissues in situ should have been removed from the serum.

In general, the conditions found at autopsy in this animal corresponded with those observed in pig 89, just described. They differed, however, in that there was more conspicuous evidence of blood destruction; in that the kidneys were involved, being of a dark chocolate color, congested, and markedly pigmented, as revealed by microscopic study; and in that the adrenal bodies presented several areas giving somewhat the reactions of hyaline tissue, though not fully characteristic. No destruction of nuclei was observed in these areas and, in general, nuclear staining differed little from normal. The cell protoplasm took up eosin more conspicuously than usual, and more so than the surrounding portions of the sections, and had the appearance of being fused together so that the outlines of individual cells was difficult and at times impossible to detect. A similar condition was seen in zona reticulosa. In this locality it was not isolated as in zona fasciculata, but was more generally diffused. While no fragmentation of nuclei was seen, still many of the nuclei were conspicuous for the unusual distribution of their chromatin. In this zone were numerous apparently degenerated and unusually vacuolated cells. The condition found in the adrenals of this animal was by no means so definite as in the preceding pig, and was manifestly of different origin. The peritoneal cavity of this animal was found to be sterile by culture tests. In both autopsies there was evidence of blood destruction in varying degrees.

VII. GENERAL COMMENTS. — If it be insisted that the serum of animals immunized by the injection of definite types of alien cells shall have a specific affinity for only those cells, that is to say, shall be possessed of only specific morphological affinities, then obviously the results here recorded are at variance with the doctrine governing our conceptions of artificially induced cytolytic sera in general. If, on the other hand, it be admitted that the specificity of these sera is chemical in its relations, then there can be no reasonable grounds for surprise that such sera occasionally manifest affinities not only for the cells with which the animal furnishing the serum had been treated, but for other elements of the body as well; for it is scarcely justifiable to assume that morphological distinction means necessarily chemical distinction throughout. In many particulars the different cells of the body, even though morphologically and physiologically unlike, possess, nevertheless, certain chemical constituents in common, and it seems not unlikely that the multiple activity or the apparent lack of strict specificity occasionally exhibited by certain of the cytolytic sera may be referable to the stimulating action of atom groups, common to many different cells, upon particular receptors likewise common to various cells for which they possess chemical affinities, resulting in the over-production in the immune animal of such receptors. In the test tube or even in the animal body these may exhibit affinities for structures of very different morphology and function from those used for immunization, but which may, nevertheless, be more or less closely allied in chemical composition.

In the experiments here recorded active hemolysis was found to be the most characteristic property of the serum of rabbits into which the adrenal bodies of the guinea-pig had been injected, and affinity for the adrenal cells was manifested only in the test tube, by the power to agglutinate adrenal cells. By the methods used lysis of the adrenal cells in the test tube was not certainly seen. This, however, may be referable to technical omissions. In the studies of von Dungern[1] and of Moxter,[2] with ciliated epithelium and testicular infusions, hemolytic activity was also exhibited by the serum of the immune animals, though in both cases the sera contained in addition demonstrable specific principles; in the one case destructive for ciliated epithelium, in the other for spermatozoa, when injected with these cells into the peritoneal cavity of the guinea-pig.

These observations led both von Dungern and Moxter to regard the serum obtained by them as not strictly specific, but rather as having affinities both for erythrocytes and for epithelium and spermatozoa respectively. Moreover, von Dungern observes that the serum resulting from immunization of animals with ciliated epithelium is equally active against the epithelium of the mammary gland,[3] both epithelial in nature it is true, but still morphologically distinct. In commenting upon these observations, both Metchnikoff[4] and his pupil, Metalnikoff,[5] maintain that von Dungern and

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[1] Münch. med. Woch., 1899, No. 38.

[2] Deutsche med. Woch., 1900, No. 4.

[3] "Die Antikarper," etc., von Dungern; Gustav Fischer, Jena, 1903, S. 30.

[4] Annales de l'Institut. Pasteur, 1900, Tome xiv, p. 4 and p. 373.

[5] Ibid., 1900, Tome xiv, p. 581.

Moxter are in error; that the hemolysis observed in these sera is referable to the blood unavoidably injected with the epithelial and testicular cells used by them. This position they endeavor to support experimentally by injecting animals with mixtures of spermatozoa and defibrinated blood. The result, as might have been expected a priori, was a serum having both hemolytic and spermotoxic activities, and the specific receptors could easily be separated or removed. In all of our own efforts to produce an actively hemolytic serum, and so far as we can learn from the experience of others, it has been necessary to use very much greater volumes of defibrinated blood than would be mixed with the infusion of an organ to be employed in the preparation of a specific cytolytic serum, and the result of such an admixture is so well known to every worker in this field that one can scarcely conceive the neglect of special effort on the part of the experimenter to avoid it. That a certain quantity of blood was injected with the adrenals in our experiments there can be no doubt, but how much it is impossible to state with even approximate accuracy. It must, however, have been very small indeed, since the adrenal of the guinea-pig, though relatively vascular, is very small, and its absolute blood content must have been scarcely considerable, and of this amount some necessarily escapes on the removal of the gland. At all events, we have under way a series of experiments to determine the minimum amount of blood necessary to induce an amount of hemolysis equivalent to that observed in our adrenal sera.

That the hemolysis exhibited by our "adrenal sera" is due to the small quantity of blood injected with the adrenal cells is to us questionable, for it was often as great and even greater than that exhibited by the blood of rabbits that had been purposely injected with relatively large doses of guinea-pigs' erythrocytes. Thus, for instance, in one of our comparative tests we found, taking the hemolytic activity exhibited by normal rabbit serum on washed guinea-pigs' erythrocytes as unity, that the serum from a rabbit that had received fifty-three cubic centimeters of defibrinated guinea-pig's blood in broken doses by intraperitoneal injection, and was bled ten days after the last injection, was twice as active; the serum from adrenal rabbit B also twice as active, and that from adrenal rabbit D was five times as active, and yet neither of the animals furnishing the "adrenal serum" could have received more than the merest fraction of the amount of blood injected into the rabbit that furnished the "specific hemolytic" serum.

VIII. STUDIES UPON THE ACTIVE PRINCIPLE OF THE ADRENAL BODIES. — In the course of his numerous contributions to the general subject of immunity and its underlying mechanism, Ehrlich has from time to time expressed the opinion that, while tolerance may occasionally be exhibited by animals to progressively increasing doses of certain definitely constituted poisons, this is not necessarily accompanied by the elaboration of anti-bodies, and cannot, therefore, in such cases be regarded as an immunity in the sense in which the word is to-day understood. This opinion has not been shaken by such experimental evidence as we have seen on the subject.

The studies that have been made upon the adrenal gland of different animals point to the elaboration of a something having at times marked toxic action, and through elaborate researches, especially of Abel, a crystallizable body of definite chemical composition has been isolated.[1]

In the light of our negative results with the adrenal glands themselves, we decided upon a study of the reactions of the rabbit to the active principle of the gland. These studies, though not yet complete, have, however, supplied information that is not without interest.

Through the courtesy of Professor Abel, to whom we here express our thanks, we had placed at our disposal a sufficient quantity of the active principle of the adrenal bodies to begin the work.[2]

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[1] For the literature upon this subject see Amberg, Arch. int. d. Pharm. et d. Ther., 1902, Vol. xi, p. 57.

[2] The experiments to be recorded were made with a single sample of the adrenal active principle, so that variations cannot be referred to variations in the material used.

It is needless at this time to enter into the details of the chemical nature of the substance supplied by Professor Abel. It suffices to say that it came to us in the form of a white amorphous powder, tolerably readily soluble in 1:1000 HCl when gently warmed, and gradually partaking of a more or less distinct violet color when exposed for a time to light and air.[1]

When used by us it was always in an acid solution made from the dried preparation not more than five or ten minutes before the injections. The convenient proportions for solution we found to be at the rate of two milligrams of the dried substance to one cubic centimeter of a 1:1000 solution of hydrochloric acid, and the mode of administration was always by direct intraperitoneal injection.

As stated, our problem was to determine first, if tolerance to gradually ascending doses of the drug is exhibited by rabbits; and second, if such tolerance is accompanied by the presence in the serum of anti-bodies having a restraining or neutralizing action upon the drug in the test tube or otherwise. Of fundamental importance to the success of such a study is a knowledge of the minimum lethal dose (M. L. D.) of the substance under consideration.

Notwithstanding a fair effort in this direction, we have found it impossible, by the methods used, to determine this factor. We have had full-grown rabbits die after so small a dose as one milligram, and have had some die and some recover after so large a dose as eight milligrams into the peritoneal cavity (see Exhibit III.).

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[1] A letter from Professor Abel informs us that the material supplied for these experiments is what he proposes to designate as " Epinephoin hydrate."

EXHIBIT III. GIVING RESULTS OF EFFORTS TO DETERMINE THE M. L. D. FOR THE ACTIVE PRINCIPLE OF THE ADRENAL GLAND.

Rabbit.	Weight.	Dose in mgs.	Mgs. per Kilo.	Result.
20	1,650	1.0	0.8	+ next morning.
21	1,400	1.5	1.1	Recovered.
24	1,400	4.0	2.8	+ 2½ hours.
32	1,830	4.0	2.2	Recovered.
33	2,230	4.0	1.8	+ 45 minutes.
34	2,600	4.0	1.6	Recovered.
35	1,820	6.0	3.3	+ 2½ hours.
60	1,870	6.0	3.2	+ 2 hours.
41	2,490	6.5	2.6	Recovered.
51	2,210	8.0	3.6	Recovered.
52	2,040	8.0	3.9	Recovered.
25	1,720	12.0	7.0	+ 2½ hours.

+ = Dead.

There is a corresponding irregularity in the manifestation of physiological results in those animals to which the dose is not fatal. Some individuals appear to be profoundly affected after so small a dose as one-half milligram, while others exhibit no inconvenience from doses of from four to eight milligrams. One of the rabbits (No. 27) to which a total of twenty-eight milligrams was given in twelve daily doses, beginning with six-tenths milligram and gradually ascending in amount to four milligrams, was as much affected after the last as after the first dose, and was usually so much affected by the injections that for a time following each injection its recovery was doubtful, while two other rabbits in the same series (Nos. 28 and 30), injected at the same time with equivalent amounts, exhibited at no time either early or late in the course of the experiment anything more than a very slight acceleration of respiration that passed off in fifteen or twenty minutes, and was often even difficult of detection.

Notwithstanding our failure to determine the M. L. D. for this substance we began a series of immunization (?) experiments to observe the changes, if any, exhibited by the blood of those animals in which we hoped to detect a tolerance to the drug. Six mature healthy rabbits were selected, regardless of weight, and each received daily intraperitoneal doses beginning with six-tenths milligram, and gradually ascending to four and six milligrams in different animals. (See Exhibit IV.) All lost weight during the course of this experiment, the loss ranging between three hundred and six hundred grams. After a total of twenty-eight milligrams one animal of the series died, with nothing at autopsy to account for death. Microscopically, the adrenals were congested, but otherwise not changed. Five days after the last injection of another rabbit, that had had twenty-eight milligrams in all, it was bled and its serum tested for the presence of antitoxic substances. The day following the last injection of another rabbit that had also had a total of twenty-eight milligrams it was bled and its serum also tested, while on the day following the last injection of another rabbit that had had a total of thirty-six milligrams, blood was drawn and tests of the serum made.

These tests were made by mixing with that quantity of the drug that had most often proved fatal (dissolved in 1 : 1000 HCl) from two to four cubic centimeters of the serum from the treated rabbits. The experiments were controlled:

By direct injections of the drug in four, six, and eight milligram doses dissolved in two, three, and four cubic centimeters respectively of 1 : 1000 HCl solution;

By the dilution of similarly dissolved doses with 0.85 per cent NaCl solution in amounts equivalent to that represented by the serum mixture. This was sometimes injected at once, and again after two hours at 38° C.,

By mixtures of similar doses with varying quantities of normal rabbit serum used at once, and after two hours at 38° C.; and

By mixing as said above, of similar quantities of the toxic substance with different amounts of the serum from the

EXHIBIT IV. — RECORDS OF RABBITS RECEIVING GRADUALLY INCREASING DOSES OF THE ACTIVE PRINCIPLE OF THE ADRENAL BODY.
ALL INJECTIONS INTRAPERITONEAL.

March, 1903.

NUMBER.	Weight.	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
26...........	2,770	0.6	1.1	1.2	1.4	1.8	2.2	2.5	3.0	3.3	3.6	4.0	4.0	+															
27...........	1,740	0.6	1.0	1.2	1.4	1.8	2.2	2.5	3.0	3.3	3.6	4.0	4.0	...	...	...	...	...	Bl.	...	...	...	...	...	...	...	...	6.0	+ in 15 min.
28...........	2,190	0.6	1.0	1.2	1.4	1.8	2.2	2.5	3.0	3.3	3.6	4.0	4.0	4.0	4.0	4.0	...	6.0	...	...	...	...	...	...	...	...	...	6.0	Recovered ϕ.
29...........	1,870	0.6	1.0	1.2	1.4	1.8	2.2	2.5	3.0	3.3	3.6	4.0	4.0	4.0	4.0	4.0	...	6.0	...	...	...	...	...	...	...	...	...	6.0	Recovered ‡.
30...........	3,420	0.6	1.0	1.2	1.4	1.8	2.2	2.5	3.0	3.3	3.6	4.0	4.0	Bl.	...	...	...	...	...	...	...	...	...	...	...	...	...	6.0	Recovered ϕ.
31...........	1,820	0.6	1.0	1.2	1.4	1.8	2.2	2.5	2.0	3.3	3.6	4.0	4.0	4.0	4.0	Bl.	...	...	...	...	...	...	...	...	...	...	...	6.0	Recovered ‡.
																								Control..			..	6.0	+ 2 hours.

‡ Nos. 29 and 31 so seriously affected that they were not expected to recover; 31 died 2 days later.
ϕ Nos. 28 and 30 scarcely, if at all, affected. No. 28 found dead 13 days later.
+ = Dead.
Bl. = Bled.

treated (tolerant) animals; used also sometimes at once and again after two hours at 38° C.

One control was made with a volume of the hydrochloric acid (1:1000) alone to observe the effects upon the general condition of the animal. These were so limited in extent and of such brief duration as not materially to interfere with the correct interpretation of the results of injection of the drug in such a solution.

It is needless here to enter into the details of these experiments. They were negative throughout in demonstrating antitoxic properties in the serum from the treated animals, and were very much confused by the frequent failure of the drug, even in six and eight milligram doses, to cause more than a transient effect in the control animals.

In a number of cases the physiological action of the drug was as marked in the animals getting the "specific" serum along with the drug, as in those getting the drug alone or diluted with salt solution. The animals that got the mixtures of the drug and normal rabbit serum were neither more nor less affected than those that received the drug and "specific" serum.

By the method of administration and observation employed in these experiments, the specific physiological action of the drug is made manifest, principally through respiratory disturbances. Within a few minutes after injection the rate of respiration is increased, and after another ten minutes has become so accelerated that it is impossible to determine its rate by counting. When in this condition, the rabbit settles upon its belly or side with its front and hind legs braced against the walls of its cage, its head thrown back, and its mouth, from which there is at times an excessive flow of saliva, partly open. It may remain in this position, if not molested, until death, or until the acute symptoms have passed off. This may be fifteen minutes or several hours, according to dose and individual peculiarities. In some cases the animal is constantly shifting its position, always giving one the impression that an effort is being made to assume a position best adapted to labored respiration. We have not seen paralysis in any of our animals; nor have we noted any affection of the bowels.

Usually there is marked urination immediately or shortly after the injections.

There has been no discharge of blood from the air passages of any of our animals.

Unless distinct physiological effects are observed within a half hour after the injection, they are not apt to develop, and in none of our animals in which there was a delay of thirty to forty-five minutes in the appearance of the respiratory symptoms was there a fatal termination, no matter what the dose may have been. In three of our control animals, ranging in weight from 1,740 to 2,210 grams, to which eight milligrams each of the drug were given, there was practically no disturbance of the respiration. On the other hand, as said, many develop alarming symptoms after very much smaller doses.

The conditions found post mortem in those that die in from two to four hours after the injection are usually confined to the thorax. The lungs are engorged with blood and the heart has the appearance of having been forcibly distended with blood.

The abdominal viscera may be normal, but are more often pale in appearance.

In none of our autopsies have we discovered evidence leading us to believe that the material was injected elsewhere than in the peritoneal cavity. Punctures of the intestines and bladder were sought for, but none were encountered.

The most impressive feature of this group of experiments is the remarkable variation in individual susceptibility to the action of the drug, for which, unfortunately, there is no ready explanation. To assume that the mechanism by which the toxicity of the adrenal secretions are normally regulated during life varies with different individuals, being adequate in the one case to meet the demands made by the sudden artificial addition of unusual quantities of this toxic principle, while in others it is not, may be reasonable and logical, but not as yet supported by established facts, and has little more than speculative value in explaining the irregularity encountered by us and others.[1]

IX. CONCLUSIONS. — The foregoing results seem to warrant the following conclusions:

I. It is doubtful if repeated injections of guinea-pig adrenals into rabbits result in the elaboration of a serum having demonstrable specific affinities for the adrenal glands of the guinea-pig in situ.

II. The most conspicuous characteristic of a serum obtained by the above plan is its destructive action upon the blood of the guinea-pig. This we do not believe is referable to the small quantity of blood injected with the adrenal cells during the immunization of the rabbit.

III. If the hemolytic receptors be removed from such an "adrenal serum" its toxic action upon the guinea-pig disappears.

IV. Rabbits exhibit more or less of tolerance to gradually ascending doses (intraperitoneal) of the active principle of the adrenal gland. This tolerance is probably not accompanied by the presence in the serum of the rabbits of substances antagonistic (antitoxic) to the adrenal active principle in vitro.

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[1] In a single experiment made with the serum of a rabbit after the intraperitoneal injection of dog's adrenals we found a slight reduction in blood pressure of the dog to follow the intraperitoneal injection of this serum. Similar injections of dogs with normal rabbit serum, with the normal sera of the duck and with the "adrenal serum" from the rabbit prepared with guinea-pigs' adrenals as above, all in doses of from ten to twelve cubic centimeters, had no appreciable effect upon blood pressure. The only significance of this observation is in connection with the negative results of the other studies here recorded. It suggests the possibility of positive results by a plan of analysis not taken up in the body of our work.

ENZYMES IN TUMORS.[1]

B. H. Buxton.

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The following is a preliminary report on a line of experimental work undertaken in connection with the Huntington fund for cancer research, and carried out in the pathological laboratory of Cornell Medical College.

My best thanks are due to many of my confrères, more particularly to Dr. Carl Beck, for material kindly provided.

I am also greatly indebted to Professors Green, of Cambridge, and Beijerinck, of Delft, both of whom very kindly gave me most valuable instruction last summer in the methods of testing for enzymes.

The object of the work is to endeavor to determine:

(1.) The nature of any enzymes present in malignant tumors.

(2.) If any particular enzymes are constantly present.

(3.) If so, are they in any way different to those found in normal tissues.

The enzymes in question would, of course, be strictly intracellular as opposed to the extracellular enzymes of the digestive tract. The extracellular enzymes have long been known and very closely studied, but until comparatively recent years, ferment action occurring in the animal cell itself was ascribed to vital action of the protoplasm. It was supposed that in this respect there is a difference between vegetable and animal enzymes: the former acting in the cell itself, whilst the latter are poured out into the digestive tract to do their work outside of the actual organism.

It gradually came to be recognized, however, that from animal cells — cells, moreover, which are not concerned in the manufacture of extracellular enzymes there can be extracted by maceration in water certain soluble principles, which can

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[1] Received for publication April 6, 1903.

exert various ferment actions altogether apart from the life of the cell.

By grinding yeast cells to powder and pressing out the juice, Buchner was able to extract zymase, the principle which converts glucose into alcohol and carbon dioxide. It has been found impossible by simple maceration of yeast to obtain any such ferment apart from the living cell. Experiments on the lines of mechanically tearing cells to pieces in various ways are being systematically carried on at the Jenner Institute in London by MacFadyean and Rowland, but it still remains an open question whether animal cells can be got to yield by such methods any enzymes which cannot be extracted by simple maceration.

The subject of intracellular ferments for normal tissues has been worked over pretty thoroughly in recent years, but as applied to pathological tissues has received but little attention. Petry[24] found that carcinoma tissue very readily undergoes autolysis, with increase of non-coagulable proteids and formation of leucin, tyrosin, hexon and purin bases at the expense of the more complex proteids coagulable by heat. The autolysis is due to the presence of a proteolytic ferment, the products corresponding to those found by Salkowski and others in autolysis of normal organs.

Emerson[10] in a study on the influence of carcinoma of the stomach on digestion, comes to the conclusion that in carcinomatous tissue there exist proteolytic ferments which carry the digestion beyond the albumose and peptone stages. Such ferments dissolved out from the ulcerated surface of carcinoma of the stomach accelerate the action of the pepsin, and their ultimate products, diamido bodies, and so forth, enter into loose combination with the HCl. In this way he accounts for the absence of free HCl in such cases. There are some weak points in his arguments, and the work needs confirmation, which Emerson says it is receiving at the hands of Dr. Falta, of Basel.

EXPERIMENTAL METHODS.

The first step to be taken was to test a number of malignant tumors to see what enzymes they contain. Since it is not always easy to obtain cancer material in sufficient quantity to make filtered extracts and test for enzymes in solution, an attempt was made to demonstrate their presence by means of diffusion in agar plates.

For this the methods adopted by Beijerinck[2] and Eijckman[8] for demonstration of vegetable and bacterial enzymes have been utilized and modified to suit requirements. It is fully realized, however, that the results obtained require checking and confirmation by means of extracts prepared and tested in the usual way, and the intention is to extend the work in this direction. If the method of diffusion in agar plates proves reliable, as there seems little reason to doubt will be the case, it will certainly be a convenient one to adopt, since very small amounts of tissue are required, and the method is simplicity itself. To quantitative determinations it is obvious that it can never be applied. At the best only rough comparisons can be made.

Whatever the method adopted, however, there will always be two great disturbing elements in examination of the cell products of tumors:

(1.) The presence of blood from which the tissues cannot be freed by the methods used in animal experiment.

(2.) The presence, especially in carcinoma, of tissue which merely represents a productive inflammatory reaction, and is not, properly speaking, a part of the tumor itself. Fully formed connective tissue contains little or no active ferment, but young connective tissue and leucocytes will always have to be reckoned with.

The methods of procedure were as follows: The tumor, or part of it, freed as far as possible from other tissues was weighed and chopped fine. About an equal amount of pumice stone was added, and the whole pounded as fine as possible in a mortar. Enough fifty per cent glycerin in water was then added to bring the mass to the consistence of a soft paste or pulp. Vernon[30] found fifty per cent glycerin the best extractive for enzymes.

The pulp was then divided into two parts, and put into two sterile bottles labelled A and B: B was boiled for twenty minutes, and a little more fifty per cent glycerin was added to bring it again to its original consistence. Twenty or thirty grams of each were found to be sufficient, and for some small tumors a good deal less had to be put up with. A little chloroform was added to each, stirred well in, and renewed from time to time as found necessary.

The tumor was at once tested bacteriologically. A loopful of the pulp was put into a melted agar tube and plated out. It may be mentioned once for all that the tumors tabulated here were practically sterile. There were in some cases a few colonies on the plates, but never more than could be accounted for by unavoidable contamination during manipulation.

After keeping for two or three weeks, the pulp became absolutely sterile, except for the occasional presence of a few spores belonging to the mesentericus or hay bacillus group. It may be taken for granted, then, that there were no enzymes due to previous infection by bacteria present in any of the tumors.

The agar used as a basis for the plates was made up to two per cent with tap water, boiled till dissolved, cleared by prolonged boiling with white of egg, and filtered through cloth and absorbent cotton. The agar was distributed into small flasks, 100 cc. in each, more or less, as required, and sterilized.

When needed for use the agar was melted and a little thymol added whilst hot, so that it would dissolve thoroughly. The agar was then cooled down gradually to 50° C. or 60° C.; the substance needed for the reaction added, and as soon as this was thoroughly mixed or dissolved, the agar was poured into Petri dishes, about 15 cc. in each, and allowed to set.

A small spoonful, about ½ cc., of the pulp A and another of B were put on the surface of the agar, spread out in a circle of 20 mm. diameter, and the plate kept in an air-tight incubator at 40° C., exposed to vapor of chloroform water. In spite of the thymol and chloroform, it was found necessary to conduct the operations as aseptically as possible, since some spore-bearing bacilli of the mesentericus or hay bacillus groups would occasionally, though not often, grow on the plates after two or three days. This was not of much consequence, however, since the observations on the enzymes seldom extended over more than forty-eight hours. Fresh milk could not be used for the milk agar plates, or there would be countless colonies in forty-eight hours. There is one advantage in using agar plates, and that is, that any contamination by bacteria can be at once detected.

EXPERIMENTS.

1. *Proteolytic enzymes.*

(*a.*) *Egg albumen.* Suspension 5 per cent of white of egg in agar. Slightly opalescent.

This has been found very useful for demonstration of bacterial enzymes. An old culture of anthrax, for example, together with some of the agar on which it has been growing, is smeared over the surface. In twenty-four to forty-eight hours a clear ring of 10–20 mm. all around the smear can be seen, and the effect is heightened by heating over a flame. The egg albumen coagulates, and the agar becomes quite opaque, except for the ring around the anthrax culture. Growth has been prevented by the thymol and chloroform, so that the change must have been due to diffusion of enzymes previously secreted.

This method applied to tumors, however, led to no positive results. In none of them could proteolytic enzymes acting on egg albumen be demonstrated.

Hedin & Rowland[15] found that intracellular proteolytic enzymes of the spleen were most active in slightly acid solutions. In view of this, and of Emerson's experiments already mentioned, egg albumen plates with .35 per cent HCl were used in some cases as well as the neutral ones, but here also the results were negative.

(*b.*) *Milk.* Agar, 90 per cent; sterilized milk, 10 per cent.

The milk plates treated with anthrax, as already described for egg albumen, show in twenty-four hours a ring of coagulation (rennet), and later on an inner clear ring due to digestion of the coagulated casein by a proteolytic enzyme. The results obtained with tumors were uneven:

Some showed no change;
Others clearing without previous coagulation;
One coagulation and no subsequent clearing;

Whilst some showed both in the clearest possible way.

None of the boiled controls had any effect on the milk.

(*c.*) *Liquefaction of gelatine.* Ten per cent water gelatine with a little thymol into Petri dishes kept at room temperature.

Here again the results were uneven. With some tumors there was liquefaction, more or less rapid, and with others none. In no case did the boiled controls have any effect on the gelatine.

Eijckman[8] found that among the bacteria those which liquefy gelatine also digest casein and vice versâ. My experiences coincide with his so far as bacteria go, but this was not always found to be the case with the tumors. No. 28, for instance, had no effect on gelatine, but coagulated and then digested the milk in a very marked way.

(*d.*) *Hemolysins.* Several experiments were made with agar + .7 per cent NaCl, in which a little defibrinated blood was suspended. In this the blood corpuscles remain intact, but are dissolved by thymol or chloroform vapor. Antiseptics, therefore, could not be used, and it has hitherto been found impossible to avoid some contamination of the plates so that the results have been negative, although there were indications of hemolysins in some instances.

(*e.*) *Fibrin.* Pounded fine and suspended in agar was never affected in the least.

(*f.*) *Serum.* Coagulated in Petri dishes was not liquefied, but only a few of the tumors were tested in this way.

The experiments with proteolytic enzymes then afforded uncertain results. The softer and more cellular tumors one would expect to contain more than the denser, more fibrous ones, but this does not always appear to be the case, although it usually is.

Petry, whose experiments on autolysis of carcinoma tissue have already been mentioned, has been quoted as saying that such autolysis proceeds more quickly than with normal tissues. But he actually only made comparisons with normal breast tissue, which is relatively poor in cells, and there seems so far no reason to suppose that carcinoma tissue undergoes autolysis more rapidly than normal highly cellular organs, such as liver.

Jacoby[17] has recently advanced the opinion that the proteolytic intracellular ferments of the various organs are to some extent specific. He found at any rate that ferments in liver extract, which have the power of reducing the amount of proteid coagulable by heat in liver tissue, have no effect on the coagulable proteids of lung tissue, although they can attack the albumoses in both instances. He points out that the coagulable proteids of lung and liver would never come in contact with each other, but the albumoses from the lung might reach the liver and other organs by way of the circulation, as has been recently shown by Embden and Knoop[9] and Langstein.[20] The former then have developed specificity, and can only be attacked by specific enzymes,* whilst the albumoses are general, and can be acted upon by enzymes common to all organs. Jacoby finds an analogy to this in the action of the complements, some of which are strictly specific, whilst others have a more general character.

Some such specificity of the proteid and enzyme molecules may, perhaps, account for the egg albumen in the agar plates always remaining untouched, whilst the non-coagulable casein and the still simpler gelatine were often digested.

This hypothesis of hyper-specificity, as it may be called, for proteolytic ferments does not, of course, apply to those of the digestive tract, but only to such as act intracellularly.

It seems, however, more probable that the proteolytic enzyme of the tumors is allied to the erepsin of Cohnheim (Zeit. Phys. Chem., Vols. 33, 35, 36, 1901 and 1902), found by him in the secretions, but more particularly in the mucous membrane of the small intestine. Erepsin cannot attack egg albumen or fibrin, but can actively reduce, not only albumoses and peptones, but also casein to crystallizable products; leucin, tyrosin, etc.

Cohnheim maintains that erepsin is an intracellular ferment which attacks the peptones at the moment of their resorption by the cells of the mucous membrane.

Amylase. — Two kinds of plates were prepared:

(1.) Agar, 100 c.c. + soluble starch .5 grams.

(2.) Agar, 100 c.c. + glycogen .25 grams.

After two days the plates were flooded with HCl 3.5 per cent for a minute or so to remove any traces of alkalinity which would interfere with the subsequent reaction. The HCl was then poured off and diluted Lugol solution was poured over the surface. The starch agar turns deep blue and the glycogen brown, except where there has been some conversion into sugar.

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* Bokorny[3] considers that in the present state of our knowledge we must assume a special plasma for each function of the cell, and emphasizes the intimate relations of the protoplasm of a cell and its enzyme.

In every case there was some conversion shown by a clear ring around A, whilst B always remained unchanged. In the tables the width of the rings is marked in millimeters in each case.

Glycogen has often been demonstrated in tumor cells by micro-chemical methods. Lubarsch[23] could only find it in sixty-two out of four hundred and eighty tumors, but Brault[4] claims that it can always be found, if small pieces of the tumor tissue are taken quite fresh and put directly into absolute alcohol, so that the glycogen has no chance to dissolve out. The more malignant the tumor, the more glycogen it contains, and prognosis can be based on the amount observable.

A reversing action for some enzymes has been demonstrated. Croft Hill showed that if maltase be added to a concentrated solution of glucose some of the latter is synthesized by dehydration into maltose.

Hanriot,[14] Loevenhardt,[22] and others have shown that lipase has a reversing action and can either synthesize or analyze fats in such a way as to maintain the necessary equilibrium in the various cells of the organism.

Cremer,[6] on adding glucose to Buchner's pressed yeast extract, found traces of glycogen were formed, but it cannot yet be considered proved that amylase is able to exert a reversing action.

That the glycogen in tumor cells is synthesized "in sutu" and not brought in as glycogen, there can be little doubt, and it is significant to find amylase so constantly present in tumor tissue. A future study will be to compare the amounts of glycogen and amylase found in tumors and see if they bear any relations to each other. Up to now the amount of amylase present, so far as can be roughly judged by width in millimeters of starch converted, does not seem to vary directly with the malignancy. The very malignant tumors of the liver contained comparatively little amylase. This again seems significant and worth further investigation, since the adult liver contains glycogen, whilst it is absent in the embryonic liver. In other tissues the reverse obtains, with the exception of the muscles which do not enter into the question so far as malignant tumors are concerned.

Lipase. — For demonstration of this in the blood and other organs, Hanriot used solutions of butyrin, and Loevenhardt of ethylbutyrate, with positive results in both instances, so far as the blood is concerned.

Doyen and Morel,[7] using emulsions of olive oil, could not confirm Hanriot's experiments, but concluded that blood contains no lipase, and that the formation of acid in Hanriot's solutions was due to bacterial growth. Arthus [1] found a ferment in blood which converts butyrin into glycerin and butyric acid, but has no effect on vegetable or animal fats. He calls it butyrase, distinguishing it from true lipase. It is a question, however, if the action be not one of degree rather than of kind. The lipase present may be sufficiently concentrated to convert butyrin, but not the more stable compounds of the higher fatty acids. An analogous instance may be found in the metacasein reaction. Roberts [26] showed that milk treated with pancreatic extract does not curdle at room temperature, but will do so on boiling. He concluded that he had demonstrated a milk-curdling ferment of a different nature to true rennet, but it is now held that it is only a question of the amount of rennet and the conditions under which it acts.[12]

The method adopted here was to mix the fatty substance with litmus agar just sufficiently alkalinized to turn the litmus blue. The lipase diffuses out into the medium, converting the fat into a fatty acid and something else; the litmus turning red on account of the acid liberated.

Neither ethylbutyrate nor oil emulsion were found to give satisfactory results; only traces of acid being occasionally formed, and in the great majority of instances none at all. It appears as if the lipase present is not strong enough to decompose the substances under the conditions offered, but the more labile butyrin (one per cent in agar) always yields positive results. In an hour or so there is distinct reddening all round A, and in five hours a red ring, varying from one millimeter in some instances to five or six in others. The reddening is progressive, spreading all over the A side of the plate in twenty-four hours.

Whether it be true lipase or not, there is certainly something in the tumors which can rapidly convert butyrin into glycerin and butyric acid, and this power is destroyed on boiling, since B never showed any traces of reddening.

Bacterial growth was excluded, and the original reaction of the pulp was either neutral or so very faintly acid to litmus that it had no visible effect on plain litmus agar plate.

It is very fascinating to watch the progressive reddening, but it must be confessed that the value of the reaction for tumors will probably prove to be nil, since normal rabbit organs, and more particularly the blood, were also found to convert the butyrin in every instance.

Suspensions of butter in agar, about two per cent, were tried for micro-organisms. No bacteria were found which could split the butter, but aspergillus niger, which is known to secrete lipase,[5] reddens the litmus all around the growth in forty-eight hours.

On applying this to the tumors it was found that in some instances there was slight reddening, but the results were uncertain and not worth recording.

Oxidases. — Animal tissues are able to readily oxidize many substances which in the chemical laboratory can only be oxidized very slowly at body temperature, or rapidly at much higher temperature. Until recently this power of accelerating oxidation was attributed to vital energy, but it is now known that it is due, partially if not entirely,[*]

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[*] Jacoby[19] points out there are many substances oxidizable in the body which ferments will not oxidize. It may, however, be only a question of time before ferments for these are discovered.

to soluble oxidizing ferments, oxidases, which can be extracted from the cell, and exert their influence altogether apart from it. The oxidases in the biological seem to play the part of heat in the chemical world.

Again there are substances, such as oxalic acid,[26] easily oxidizable by chemical methods, which pass through the body unchanged, and are therefore not affected by the oxidases.

The oxidases, then, can exert a selective action, presumably oxidizing such substances as in their oxidized condition will be of use to, or less harmful to, the cell, whilst those which are indifferent or naturally foreign to the organism are not affected.

The individual oxidases do not appear to possess specificity. Those which respond to one reaction will, as a rule, respond to others, indicating that they can oxidize more than one particular substance, but there are certainly different kinds. A ferment can be extracted, for instance, from liver tissue which does not give the guaiac reaction, but can oxidize salicyl aldehyde to salicylic acid.[29]

The oxidases, like other ferments, are destroyed by boiling. There are several reactions by which their presence may by recognized.

1. The Guaiac test. (*a.*) To a little distilled water in a tilted porcelain dish a saturated alcoholic solution of gum guaiac is added to the point of a milky emulsion.

A small quantity of the tumor pulp is placed at the margin of the fluid, and a blue color due to oxidation of the guaiaconic acid contained in the guaiac, would show the presence of oxidase. In no case did the blue color appear. Oxidases which turn guaiac blue then were uniformly absent, nor were they ever found in normal organs, human or animal. The latter observations agree with those of Rosell,[27] who in normal organs never found oxidases turning guaiac blue. (*b.*) On adding a little H_2O_2 to the above after thirty minutes or so, a blue color always appeared, more or less rapidly diffusing throughout the emulsion. Peroxidases, therefore, were always present, and the same holds good for normal tissues. Blood, as is so well known, gives the reaction very readily, and its presence in the tumors no doubt accelerates the action, though Jacoby[18] says tissue freed from blood is also active.

This property of turning guaiac blue in the presence of H_2O_2 was greatly weakened, though not entirely lost, on boiling, so that there must be something present besides the peroxidases which can oxidize the guaiac. Schaer,[28] indeed, found that there are a great many such substances.

H_2O_2 ferment (Catalase). All the tumors decomposed H_2O_2 with release of oxygen, and this appears to be a property of all living protoplasm. Boiling invariably destroyed the activity.

This ferment is probably identical with peroxidase, the liberated oxygen in the presence of guaiac having the power of oxidizing it.

Indo-phenol reaction. — Freshly dissolved dimethylparaphenylendiamin and *a* naphthol were mixed in equal parts and 6–8 cc. added to each 100 cc. of agar, at once plated out, and the tumor pulp placed on the surface. In twenty-four hours such plates have become pale blue all over (indophenol), but where the reaction has been accelerated and intensified by oxidizing ferments, as is always the case with a slice of potato, there is very distinct deep blue ring all round the substance. The tumors never afforded such clear deep blue rings as the potato, and the reaction often failed to materialize at all, so that the results so far obtained do not seem to point to any conclusion. Rosell observed this reaction with some normal organs and failed with others.

Lillie by micro-chemical methods demonstrated the indophenol reaction in leucocytes and other cells, the blue color appearing most intense in the neighborhood of the nuclei.

Hydrokinone reaction. — Agar, 100 cc.; hydrokinone, 1 gram. In solutions containing oxidases the hydrokinone is gradually oxidized to the dark reddish quinone and after a

TUMORS.	PROTEOLYTIC.						AMYLASE.				LIPASE.		OXIDASES.			
	Milk.				Gelatin.		Starch.		Glycogen.		Butyrin.		Hydrokinone.		Indophenol.	
	Coag.	mm.	Clear.	mm.	Liq.		Clear.	mm.	Clear.	mm.	Red.	mm.	Dark.	mm.	Blue.	mm.
1. Carcinoma breast scirrhous	.....	.....	.....	.....	+	.....	+	5	+	2	.....	.....	+			
2. " "	—	.....	—	.....	—	.....	+	5	+	3	+	10	+	2–3		
3. " " scirrhous	—	.....	—	.....	—	.....	+	5	+	2–3	+	10	+	2		
4. " " cellular	—	.....	—	.....	—	.....	+	8	+	6	+	10	+	slight	—	
5. " lymph nodes of No. 4	.....	.....	+	trace	—	.....	+	7	+	4	+	12	+	slight		
6. " breast scirrhous	+	4	+	2	+	slight	+	5	+	3	+	10	—	.....	—	
7. " "	.....	.....	+	slight	+	slight	+	3–4	+	slight	+	3–4	+	trace	trace	
8. " " scirrhous	.....	.....	+	3–4	—	.....	+	10	.....	.....	+	6	+	trace	+	
9. " " "	+	1	+	4	+	slight	+	10	+	5	+	10				
10. " " cellular	+	5	+	3	+	much	+	8	.....	.....	+	10	.....	.....	+	2–3
11. " " scirrhous	—	—	—	.....	+	.....	+	4	+	3	+	12	+	5		
12. " liver	.....	.....	+	3	—?	.....	+	5	—?	.....	+	15	+	5	+	?
13. " "	+	3	+	3	—	.....	+	5–6	—?	.....	+	20	+	slight		

14. " "	+	5	+	3	—	.....	+	5	+	2–3	+	much	+	5	+	slight
15. " "	+	5	+	2	+	much	+	5	+	little	+	much	+	4–5	+	
16. " "	+	5	+	5	+?	.....	+	4–5	.....	.....	+	much	.....	.....	+?	
17. " bladder	—	.....	—?	.....	—	.....	+	5	.....	.....	+	15	+	slight		
18. Epithelioma tongue	.....	.....	.....	.....	—	.....	+	.....	+	.....	.....	.....	—			
19. " inf. maxilla	.....	.....	.....	.....	.....	.....	+	5–6	+	5	+					
20. " same as 19	—	.....	+	5	+	much	+	7	+	5	+	10	+	5	—	
21. " lip	.....	.....	.....	.....	—	.....	+	5	+	2	+	8	—			
22. "	—	.....	+	4	+	.....	+	4	+	4	+	12	+	2–5	+	slight
23. " orbit	—	.....	+?	2–4	+	much	+	7	+	2	+	8	+	3–4	+	2–3
24. Sarcoma melano	—	.....	—	.....	+	.....	.....	.....	+	2–3	.....	.....	+			
25. { Sarcoma spindle (outer part)	.....	.....	+	trace	—	.....	+	slight	+	slight	+	8	+	3–4	+	1
26. { " " (inner part)	.....	.....	+	slight	—	.....	+	slight	+	?	+	10	+	4	+	1
27. Sarcoma angio	+	6–7	.....	.....	+	much	+	5	.....	.....	+	much	—	.....	+	slight
28. " pectoral	+	1	+	3	—	.....	+	6	.....	.....	+	10	+	2–5	—	
29. " periosteal	—	.....	—	.....	—	.....	+	7	+	4	+	14				
30. Tumor of parotid	—		—		—		+	6	+	7	+	10	+	2		

time green crystals of quinhydrone separate out. On agar plates the green crystals were never observed, but potato in a few hours becomes surrounded by a dark ring. The tumors never showed this so clearly as the potato, and the results were irregular and unsatisfactory.

Besides these, a few experiments were made on agar plates with indican, changed to indoxyl by indiesac,[2] the indoxyl being oxidized by free oxygen to indigo blue; and tyrosin, blackened by tyrosinase,[11] but the results were entirely negative.

Experiments with oxydases have, therefore, led to no particular results, but so little is known of these ferments and the necessary conditions for their action, that this was only to be expected at first.

The idea of suboxidation as a cause of tumor formation seems to be gaining ground, but so far is purely speculative, having no experimental basis on which to rest. With this idea in mind, however, an extended study of the oxidizing ferments found in tumors, and a comparison of them with those of normal tissues, may help to confirm or overthrow the hypothesis.

The accompanying tables show in detail the results obtained. Tumor pulps kept for a month or two months, and tested a second and third time, never showed more than trifling variations from the primary observations either in quantity or in quality of enzymes, so that their variations have not been taken into account in making up the tables.

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A PRELIMINARY COMMUNICATION ON THE ETIOLOGY OF VARIOLA.[1]

William Thomas Councilman, George Burgess Magrath,
Walter Remsen Brinckerhoff.

It has been thought best to present this preliminary statement of certain observations which have been made in the course of an investigation on the pathology of variola. The investigation was made possible by the liberal and enlightened policy of the health department of the city of Boston, which gave the investigators access to material for anatomical and histological study, and opportunity for the clinical study of the disease. Our thanks are in the first place due to Dr. S. H. Durgin and his associates in the health department. A full report on the disease, with detailed descriptions of the lesions, will be the subject of a later publication.

In 1892 Guarnieri described in the lesions, both of vaccinia and of variola, certain peculiar inclusions in the epithelial cells. These inclusions, regarded by Guarnieri as living bodies, have been studied by many investigators, and a variety of opinions formed concerning them. They have been regarded as organisms, as leucocytes, or fragments of leucocytes, which have become included in the epithelial cells, and as products of degeneration peculiar to the disease. The most valuable work on the matter has been done by Wasielewski, who regarded the inclusions as organisms. The life cycle of the supposed organisms was not established, and the investigations up to the present have not presented sufficient evidence for the acceptance of these bodies as living.

In the lower layers of the epithelial cells of the skin, before there is any anatomical evidence of vesicle formation, there are found small, structureless bodies, from one to four microns in diameter. The epithelial cells at this time present

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little or no evidence of degeneration. The bodies, one or more in number, lie in vacuoles in the cells. The vacuoles are at first but little larger than the enclosed bodies. The bodies increase in size, and evidence of structure, consisting of granules more distinctly stained and lying in definite spaces, begins to appear. With the increase in size of the body, the vacuole of the cell enlarges until a large central space around the nucleus is formed. At this time there is but little evidence of degeneration of the nucleus. The body continues to increase and becomes granular, the granules lying in a reticular structure. The form of the larger bodies is irregular and suggests an ameboid character. They may become as large as or larger than the nucleus of an epithelial cell. A definite nucleus has not been made out in them, unless the reticular structure which stains more intensely than the rest of the body be so considered. At this time segmentation takes place, leading to the formation of small, round bodies about one micron in diameter. All this is best seen in the acute hemorrhagic cases. It may also be seen in the advancing edge of a young vesicle, the bodies becoming larger and more definite in structure as the formation of the vesicle advances. The bodies in the cells we regard as living organisms, and the gradual growth and final segmentation as a cycle in its life history. Up to this time the nuclei, although showing such evidences of degeneration as the massing of chromatin in the periphery, are not greatly altered.

At the period of segmentation, and when most of the intracellular bodies have disappeared, small round or oval ring-like bodies appear in the nucleus. These increase in size and acquire a definite structure, consisting of a series of vacuoles around a large central vacuole. The rim of the central vacuole stains more distinctly than do other parts of the body. One or more of these bodies may appear in a single nucleus. When several are present they are smaller, but have the same structure. The bodies become larger, the nuclear rim grows less distinct, and finally disappears, and the body lies in a completely degenerated cell, or this breaks down, setting free the body. With the growth and development of the intranuclear body, the vacuolar structure becomes less evident, and finally a structure is formed which contains numerous fine vacuoles. At this time small circular bodies begin to appear in it, and groups of these are surrounded by a faint ring, which probably represents the remains of the body in which they were formed. The circular, ring-like bodies have a central dot. They are one to one and a half microns in diameter, and are seen with great difficulty. They were first found in a photograph of the tissue. Single ones and clumps are also found.

We regard this intranuclear body as a further stage of development of the intracellular body, and as representing a second complete cycle of development. It develops from the spore-like bodies produced by the segmentation of the intracellular body, which pass into the nucleus. The spores which are formed from its segmentation we consider the true infecting material of variola. In a case of hemorrhagic small-pox we have found bodies similar to these spores in a blood vessel of the skin.

In vaccination of the rabbit and calf, Doctor Tyzzer, who has been following this line of investigation, has found bodies similar to those met with in the first cycle of the small-pox organism. The complete cycle of this organism, which corresponds to the primary cycle of the small-pox organism, has been traced. Doctor Tyzzer has never found any of the *intranuclear* forms. The inoculation of the rabbit, with contents of variola pustules, gives rise to bodies similar to those in vaccinia and in the first cycle of variola. Here, also, it has not been possible to follow the complete cycle. The *intranuclear* forms have not been found, although carefully sought for. Inoculation of the monkey, with contents of variola pustules, produces lesions which we must regard as variola, and in these the *intracellular* and the *intranuclear* cycles are found, occurring in the same sequence as in man. From this we regard it as extremely probable that in small-pox the complete development of the parasite through two cycles takes place, and that in vaccinia the primary cycle only. Definite conclusions on this, however, can be reached only by further study of vaccinia in animals subject both to vaccinia and to variola. The variola spore, in such an animal as the calf, finds conditions which allow of the development of the primary cycle only. The spore-like body, which is formed in this cycle when introduced into an unprotected human being, goes through the same development, producing vaccinia.

In variola the entire process of development of the parasite is concluded with the formation of the young vesicle. The spores are present in the contents of the vesicle and of the pustule, but their recognition among the detritus of cells and exudation is impossible unless differential methods of staining them shall be discovered. We have also seen forms of the intranuclear bodies, which seem to show that the intranuclear cycle is sexual in character.

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HODGKIN'S DISEASE. — A PATHOLOGICAL ANALYSIS OF NINE CASES.

CHANNING C. SIMMONS.

(From the Surgical-Pathological Laboratory, Massachusetts General Hospital.)

(For the Division of Surgery of the Medical School of Harvard University.)

The following paper is a report of nine cases of Hodgkin's disease, in which pathological examination of the tumors was made. Eight of the cases were treated at the Massachusetts General Hospital and are included in this report through the courtesy of the visiting staff. Case IX. was a private patient of Dr. C. L. Scudder and is included with his permission. The author also wishes to express his thanks to Dr. William F. Whitney and Dr. J. H. Wright for many valuable suggestions.

Considerable misunderstanding seems to exist about the disease, and many do not seem to realize that it is a definite pathological process. Much of this confusion is undoubtedly due to the numerous synonyms, some, but by no means all, of which are the following: Pseudoleukemia (Cohnheim), malignant lymphoma (Billroth), Hodgkin's disease (Wilks), lympho-sarcoma (Virchow), adenie (Trousseau), desmoid carcinoma (Schulz). Of these names, lympho-sarcoma has probably occasioned the most confusion, as it suggests a sarcoma composed of lymphocytes, while in reality Hodgkin's disease is not a malignant growth, in the sense that it infiltrates, and is not composed exclusively of lymphocytes. It seems best, until a name has been evolved describing the pathological process, to call it Hodgkin's disease.

It was first described by Hodgkin in 1832,[1] who reported seven cases, several of which, however, were probably leukemia, syphilis, or tuberculosis. His paper was forgotten until Wilks[2] brought the subject up again in 1856 and 1865. Since then much work has been done, and the histological appearances of the glands described by many men, chief among whom may be mentioned Sternberg,[3] Fischer,[4] and

Goldmann.[5] The last important publication is by Reed,[6] who gives an exhaustive review of the subject and reports eight cases with their pathological findings.

Although the pathology is recognized, the etiology is very obscure, it having been classed as an infectious disease, as a form of true leukemia, and as sarcoma.

Leukemia. — Pinkus[7] mentions lymphocytosis in the blood as a common feature of the affection, and other observers have collected series of cases showing on examination of the blood all grades between the normal and true leukemia. The histology of the glands, however, in the two affections seems to be entirely different, and the blood when examined in this series of cases showed no signs of leukemia.

Sarcoma. — In some respects it does resemble a sarcoma, but it differs from a malignant growth in that only one kind of tissue is attacked — the lymph-adenoid — and it does not infiltrate, but it tends to extend from one group of glands to those in contiguity. The metastases in the liver, lungs, and other places can be explained as the disease attacking the small amount of lymphoid tissue normally present in those organs.

Infectious diseases. — Clark[8] in summing up his paper says that it resembles an infectious disease in that there are general symptoms with fever, it may run an acute or chronic course, it may remain local for some time and then become general, and the glands first involved are usually those nearest the skin. As regards culture experiments, Verdelli[9] has collected twenty-four cases where bacteria were present in the blood at some stage of the disease, and other cases have been reported, but there is no uniformity in the observations. Delbet[10] claims to have caused the disease by inoculating a dog with a bacillus obtained from the blood of a patient suffering from Hodgkin's disease, but his proof is not convincing, and the experiment has never been repeated.

The etiological theory that seems to have the greatest number of supporters is that the disease represents an atypical form of tuberculosis. Sternberg is the main exponent of this view, and has reported a series of thirteen autopsies on cases where the clinical diagnosis of Hodgkin's disease was made, in which series he found tuberculosis present in eight. Crowder[11] has demonstrated tubercle bacilli in glands which histologically had the appearance of Hodgkin's disease. Other men, while admitting the common occurrence of tuberculosis in the disease, consider it as a secondary infection which might well be expected to occur in debilitated patients (Fischer, Goldmann, and Reed). Reed, although demonstrating in one of her cases tubercle bacilli in a typical Hodgkin's gland, believes that the two processes were distinct, but co-existent.

The conclusions reached from the examination of this series of cases agree with those of the last-mentioned observers. It was impossible to demonstrate tubercle bacilli in any of the glands examined, nor could any be found in the glands or organs of the animals inoculated with pieces of the tissue. Tuberculosis, unrecognized clinically, was present in two of the three cases which came to autopsy, but the histological picture of the two processes had nothing in common. No gland examined was affected with both tuberculosis and Hodgkin's disease.

There is general uniformity in the description of the gross pathological findings of the various observers. There is an increase in size of the lymph nodes, those of the neck being usually first affected, which, although they may become enormously enlarged, do not infiltrate, but remain discrete. The lymph glands in all parts of the body may become affected, the disease, as a rule, extending from one group of glands to those in contiguity. On section, these glands are usually described as soft, of a grayish-white color, and homogeneous, and have an almost characteristic bulging appearance. Some are hard and on section dense and fibrous. As the process advances, metastases may be found in any of the organs, where they have the same general appearance as the glands, but are of irregular shape and infiltrate the tissue. They may be found almost anywhere in the body. The spleen may or may not be affected and enlarged. Tuberculosis is often present in some organ of the body.

MICROSCOPIC EXAMINATION OF SPECIMENS FROM THE NINE CASES.— The specimens were all hardened in Zenker's fluid and embedded in paraffin. They were stained with hematoxylin and eosin as a routine, and all were also stained with carbol fuchsin, in an endeavor to demonstrate tubercle bacilli, twenty-five per cent nitric acid and ninety-five per cent alcohol being used as a decolorizer. In most cases specimens were also stained with Mallory's connective tissue stain, while in many some slides were stained with iron hematoxylin and orange G.

The examination showed in the main the same histological features as have been described by many other observers. The process consists essentially of a proliferation of the reticulum, with a resulting marked increase of fibrous tissue. Reed describes the earliest change as a proliferation of the endothelial cells covering the reticulum and of the cells in the center of the follicles. These cells infiltrate the lymph spaces and soon make the follicles indistinguishable, entirely destroying the normal appearance of the glands. In none of the specimens examined was the process in as early a stage as this, and in none could the follicles be distinguished, the lymph spaces being filled with large epithelioid cells and lymphocytes. As the process advanced, there seemed to be a very marked increase of the reticulum, with a corresponding decrease in the cellular elements, until a dense tumor was formed, consisting mainly of fibrous tissue in which a few small cellular islands could be distinguished. Macroscopically these glands were composed of bands of dense fibrous tissue separating small islands of more or less translucent gray color. The size of the gland had no relation to the stage of the disease, and, clinically, fluctuated markedly.

The lymph spaces were usually filled with cells. The vessels in the more cellular specimens were dilated, and the connective tissue seemed rather more abundant around them than elsewhere. In the fibrous specimens the vessels were decreased in number. Necrotic areas of varying size were present in nearly all the specimens, and in these the nuclei showed fragmentation, and polymorphonuclear leucocytes were present. The large epithelioid cells were usually more abundant around these areas than elsewhere.

Cellular elements. — The specimens were composed mainly of the larger and smaller forms of lymphoid cells and large epithelioid cells more or less differentiated. The derivation of these cells was not clear, but many seemed to be intimately associated with the reticulum. These epithelioid, or endothelial, cells varied greatly in appearance, but were all apparently derived primarily from the same cell, as all grades of variation could be seen between the two extremes. They were found in varying positions, some loose in the tissue, while others seemed to have marked protoplasmic processes and were connected closely with the reticulum. In one form the cell was oval, with considerable clear staining protoplasm and one vesicular nucleus containing one or more large nucleoli. This form may be considered nearest to normal, as somewhat similar cells were seen in a hyperplastic lymph gland. In some specimens many of these cells showed mitotic figures. Others were irregular in shape, and contained one or more round, oval, or irregular nuclei, sometimes centrally, at others peripherally, placed. In these cells the cytoplasm was of relatively small amount. In some it was granular, while in others it appeared to have protoplasmic processes. Vacuoles were often seen in these cells.

Giant cells were present in varying numbers in all the specimens, and varied greatly in size, some of them being as large as forty-five micro-millimeters in diameter. They were of irregular shape, and contained from two to five, or more, vesicular nuclei, having no particular arrangement. In some the nuclei were much smaller than in others, and the cytoplasm darker. Vacuoles were often seen in these cells. The giant cells did not resemble in any way the typical ones of tuberculosis. Giant cells somewhat resembling these were seen, however, in an affected gland from the neck of a case of lympho-sarcoma, the term being used in the restricted sense primary in the tonsil. The tumor occurred in one tonsil, which was removed, and six months later in the other. The gland examined was from the neck, and was removed three years later, at which time the glands and muscles were infected. These differed in that they were phagocytic, nearly all containing red blood corpuscles, while none were ever seen in the giant cells in this series of nine cases. No giant cells were seen in two cases of lymphatic leukemia, one case of mycosis fungoides, or in two cases of multiple small round-cell sarcoma of the skin.

Lymphoid cells were present in all the specimens, and in most were the commonest cellular element. Both the larger and smaller types were present.

Eosinophiles were present in varying numbers in seven out of the nine cases examined. Goldmann first called attention to these cells, and in an examination of lymph glands from thirty cases of various other diseases only found them in two — in a case of erysipelas and in one of salpingitis. In the specimens examined they varied greatly in number. In Case IX. they constituted nearly half the cells seen in some fields. They also varied in different glands from the same case.

Degenerating cells, represented by cells with dense nuclei and deeply staining protoplasm, were seen in all specimens in varying numbers. Plasma and mast cells could be found on search, but were never present in striking numbers.

ANALYSIS. (See table.) Of the nine cases examined, three came to autopsy (Cases I., III., IV.). Case VIII. died, but no autopsy was allowed. Of the remaining five, three have been lost track of (Cases II., V., and VI.), one is slowly progressing (Case VII.), and one is as reported, too short a time having elapsed since the examination for further symptoms to develop.

Of the cases, seven were in adults, two in males, and five in females, and two in children, one boy and one girl. This is rather unusual, as statistics show the disease more prevalent in young males. In six cases the symptom for which the patient sought medical advice was swelling of the glands in the neck. In two cases (Cases III. and IV.) the glands chiefly involved were post-peritoneal, but they were also present in the neck. In one (Case IX.), the mediastinal.

Tuberculin was given three times, twice with a negative result (Cases III. and. IV.), and once with the positive (Case VIII.). In one of the cases (Case III.) with a negative reaction tuberculosis was found present at the autopsy. Tuberculosis was present in some part of the body in two of the three cases that came to autopsy. These two cases, together with Case VIII., which clinically showed tuberculosis of the lung, and also gave a positive test with tuberculin, make tuberculosis positively present in three of the nine cases.

A differential blood count was unfortunately only made in six of the cases, and in most of these only once (Cases III., IV., VI., VII., VIII., and IX.). In none did it show any evidence of leukemia. Where a leucocytosis was present it was due to the polymorphonuclear neutrophiles, and usually could be accounted for.

No conclusions as to the changes in the bone marrow can well be drawn, as it was only carefully examined in one case.

Subcutaneous inoculation of animals with pieces of the glands was done in five cases (Cases IV., VI., VII., VIII., and IX.), with negative results as regards tuberculosis.

Eosinophiles were present in the glands in varying numbers in seven cases, being absent in Cases I. and IV.

From the analysis and from the microscopic examination of these cases, there seems to be no reason for classing the disease as a form of leukemia, as the blood examinations were all negative as regards that disease, and the histological changes in the glands in the two affections have practically nothing in common. Furthermore, if the lymphocytes originate in the glands, there is no reason to expect an increased number of these cells in the blood, as the change is essentially a fibrous one, and not an increase in the cellular elements or "hyperplasia," as it is often erroneously termed.

As regards tuberculosis, as has been said before, all attempts to demonstrate the bacilli, either by histological methods or by the inoculation of animals, failed.

The presence of eosinophilic leucocytes in the glands is suggestive, and in doubtful cases, particularly in the later or fibrous stage of the disease, may be of assistance in diagnosis, but is by no means to be relied upon.

CONCLUSIONS.

1. Hodgkin's disease should be regarded as an entity, and presents a definite histological picture.
2. It has no relation to leukemia or tuberculosis, although the latter may be co-existent.
3. Eosinophiles are often though not always present in the glands.

	M.	F.	Child.	Tbc.	Inoculation.	Autopsy.	Eosinophil.	Blood as at Leuk.	Glands first noticed in
Case I.	......	+	......	....	....	complicating tbc.	none	....	neck.
Case II.	......	+	......	negative	....	....	some	....	neck.
Case III.	+	......	......	negative	....	complicating tbc.	marked	negative	mainly post-peritoneal, present in neck.
Case IV.	......	+	......	....	negative	no tbc.	none	negative	mainly post-peritoneal, present in neck.
Case V.	......	+	+	....	....	....	some	....	neck.
Case VI.	......	+	......	....	negative	....	some	negative	neck.
Case VII.	+	......	......	....	negative	....	marked	negative	neck.
Case VIII	......	+	......	positive	negative	....	marked	negative	neck.
Case IX.	+	......	+	....	negative	....	extreme	negative	over sternum.

The following are the histories and pathological findings of the cases in abstract. To avoid useless repetition, the histology of the specimens is not given in full, but only the more important points noted. In all, cells of the different types described were seen in varying proportions.

Case I. — Dr. F. C. Shattuck. Female, 24, single. Dec. 21, 1901.

Family history not important. Previous history, always in good health, except an occasional sore throat.

Present illness. — Two months ago "caught cold," which left her with a hacking cough without sputum. Two weeks later left side of neck became somewhat swollen; shortly after, right side, which soon exceeded the left in size. Swellings slightly tender, and at times painful. Two weeks before date complained of some difficulty in swallowing and dyspnœa. Has lost considerable strength during last month.

Physical Examination. — Well-developed and fat. Eyelids puffy. Throat injected. On each side of the neck and in the posterior triangle is a smooth, soft swelling, reaching from the clavicle to behind the ears. Circumference of the neck 16.5 inches. No discreet glands felt. No glands in the axillæ or groins. *Chest:* Superficial veins dilated. A few fine rales heard at apices front and back. On percussion, heart and liver dullness normal. From the base of the heart marked dullness extends to the sternal notch and for .75 of an inch to the right and two inches to the left of the sternum. *Heart:* Sounds normal. *Abdomen:* Normal. *Spleen:* Not enlarged to percussion or palpation.

Urine not remarkable.

Blood. — Hemoglobin, 90 per cent. Whites, 8,000. No red or differential count made.

Temperature during stay in hospital was irregular, varying between 100° and 103° C. Pulse and respiration not remarkable.

After admittance to the hospital patient grew worse with increasing dyspnœa and cyanosis, and died Jan. 1, 1902.

Autopsy. — Anatomical diagnosis. Round-cell sarcoma of mediastinum with metastases in pericardium, liver, kidney, and lung; tubercular ulcerations in intestines; tuberculosis of the Fallopian tubes, mesenteric, lymph glands, and kidney; polyp of intestine with tubercular base undergoing adeno-carcinomatous change.

Well-developed and nourished. Conjunctival edema. Muscles pale. No fluid in peritoneal cavity. *Liver:* Four fingers' breadth below costal margin and surface presented gray-white adhesions to the diaphragm. *Spleen:* Also presented adhesions. On the liver in several places were several dense, white areas which showed through the capsule. These on section were firm, white nodules a few millimeters in diameter. Similar nodules were present in the substance of the organ. No nodules in spleen or adrenal. *Kidneys:* Combined weight two hundred and seventy-four grams. In one, surface presented three gray, slightly bulging areas, the largest two centimeters in diameter. On section these extended into the tissue as masses of gray-white new growth, with sharply marked borders.

A similar mass in the substance of the organ. At one pole of the other kidney were three cysts, the largest two centimeters in diameter. One contained clear fluid, while the other two had fibrous walls with yellowish areas in them, and contained thick, yellowish-white pus. *Tubes:* Slightly thickened, and walls showed minute yellowish areas. Peritoneum over these whitish and thickened. Other pelvic viscera normal. *Intestines:* From forty-two centimeters above to the ileo-cecal valve were nine places where the walls were thickened and the lumen constricted. The peritoneum over these thickened and showed yellowish nodules. At these places were one or more irregular moth-eaten ulcerations in the mucosa, from the base of which yellowish pus could be expressed. *Mesentery:* Thickened and showed glands with yellowish-white areas on section. *Lungs:* Bound to chest wall everywhere by adhesions. In the upper portion of the thorax was a new growth adherent to the pericardium, extending around the heart and cementing the pericardium and pleuræ together. It measured roughly eleven by fifteen centimeters. Extended upward slightly beyond sternum and pressed on great vessels, bronchi, and trachea. The growth on section was firm, lobulated, and of a grayish-white color, with a few yellowish areas. The pericardium was thickened, and showed numerous whitish nodules on its inner surface. On the visceral pleura were several whitish areas. No nodules seen in the lungs.

Microscopic examination of the lobules of growth from mediastinum showed it to consist mainly of lymphoid cells and large irregular cells with vesicular nuclei, and a small amount of protoplasm. There was a marked increase of the reticulum in comparison with that of a normal lymph gland, and in places dense bands of fibrous tissue divided the more cellular parts of the specimen. In the larger cells the nuclei were of irregular size and shape. The lymphoid cells were of the common type. Besides these, there were many large cells, with two or more vesicular nuclei and a greater amount of protoplasm. Some of these showed mitotic figures. Occasional giant cells with many large nuclei were seen. No eosinophiles seen in any section. Scattered throughout the section were a few necrotic areas infiltrated with leucocytes. *Lung:* Scattered through a good-sized section large areas of lung tissue were seen infiltrated with cells similar to those in the mediastinal tumor. Otherwise the lung showed edema and considerable desquamation of the epithelium. *Kidneys:* Section through the tumor showed the character of the growth to be the same as that in the mediastinum, with the exception that there was comparatively little fibrous tissue. The cells were of epitheliod type for the most part, and giant cells were fairly numerous. The nodule was not sharply separated from the renal tissue and faded gradually into it. Otherwise the kidney was not remarkable. A section through the wall of one of the cysts of the other kidney showed it lined with granulation tissue in which many epithelioid cells were present. Several Langhans giant cells were seen The histological picture was that of undoubted tuberculosis. The interstitial tissue of the kidney in the neighborhood was increased. Sections of the intestine and Fallopian tubes also showed undoubted tuberculosis. Section of the polyp showed beginning adeno-carcinoma and tuberculosis.

No inoculation experiments were made.

CASE II. — Dr. E. G. Cutler. Female, 26, single. March 31, 1902.

Family and previous history not remarkable. Present illness: one year ago had a cold followed by dry, hacking cough which has persisted until the present time. Shortly after noticed some lumps in the right side of the neck, which were removed in a local hospital nine months ago, but they recurred shortly after the operation. At this time a severe pruritis developed, which has persisted until the present time, causing insomnia. Lately much dyspnea and pain in the neck. Has been hoarse for the last month. Has lost some strength and weight.

Physical Examination. — Well-developed and nourished. Body covered with excoriations. Color and eyes normal. In posterior triangle of neck on both sides are many discrete glands varying up to, roughly, five centimeters in diameter. These are not tender or painful and are freely movable. Paralysis of both vocal cords, more especially right, due probably to pressure on recurrent laryngeal nerve. Right arm slightly swollen and cyanotic. Small gland felt in right axilla. None in other axilla or groins. *Chest:* On percussion dullness in front extends from base of the heart to the clavicles and one inch to the right and one to the left of the sternum. Heart apex, fifth space nipple line. Soft, systolic murmur heard at apex, transmitted into axilla; pulmonic second accentuated. Over dull area in front breathing high-pitched and expiration prolonged. Elsewhere normal.

BLOOD. — Hemoglobin, 85 per cent. Whites, 11,000. No red or differential count made.

Urine normal.

Temperature, somewhat irregular, between normal and 100° C. Pulse and respiration not remarkable.

Tuberculin given April 3, with negative result.

A gland was removed from right side of neck April 3 for diagnosis. Patient was discharged June 20, 1902, in practically the same condition as at entrance.

Pathological Examination. — The specimen consisted of an oval tumor three centimeters in its longest diameter, covered with a capsule of fibrous tissue. On section it was bulging, soft, of a grayish-white color and semi-translucent. A few ill-defined connective tissue trabeculæ could be seen running through it.

Microscopic examination showed no structure of the normal lymph gland. In no place could the follicles be distinguished. It was divided into lobules by dense bands of fibrous tissue. Between these the growth consisted mainly of lymphoid cells, but there were many larger cells with vesicular nuclei and a small amount of protoplasm. In one part of the specimen, in addition to these, were larger numbers of still larger cells with considerable protoplasm, and one or more nuclei. These often showed mitoses, and seemed to occupy often the position of the lymph sinuses, but occurred anywhere in the tissue. Others appeared intimately connected with the reticulum. Giant cells were common. The reticulum was increased, but not to the extent seen in some of the other cases. A small necrotic area was present, in one place infiltrated with lymphocytes. Around this the epithelioid and giant cells exceeded in number the lymphocytes. A few eosinophiles were seen. (Plate XV., Fig. 1.)

CASE III. — Dr. E. G. Cutler. Male, 48, married. Dec. 20, 1901.

"Quinsy sore throat" for five successive years. Two years ago caught cold, and for one month had chills and fever occurring fairly regularly every second day. This was followed by anorexia, loss of weight and strength, which has persisted until the present day. Never had any definite symptoms. Has been growing weaker and not able to work for six months. For last three weeks has been considerably worse and has noticed some edema of the ankles and dyspnea on exertion. Slight cough recently.

Physical Examination. — Fairly-well nourished. Skin pale and dry, and some brownish pigmentation on the back. A few small discrete glands felt in the posterior triangles of the neck on both sides, and also in the axillæ and groins. *Heart* and *lungs* normal. *Abdomen:* Spleen not enlarged. *Liver:* Normal. An indefinite tumor mass felt in the epigastrium. Stomach capacity seventy-two ounces. Examination of the sputum failed to show tubercle bacilli.

Tuberculin given January 19 with negative result. The spleen was slightly enlarged on February 19, and an indefinite mass palpable over the right kidney. The patient died April 2, 1902. During the stay in the hospital he failed slowly, having no definite symptoms, or changes in physical signs.

The temperature was very irregular, between normal and 103. Pulse and respiration not remarkable.

Urine not remarkable.

BLOOD. — December 24. Hemoglobin, 75 per cent. Whites, 13,600.

Polymorphonuclear neutrophiles		62	per cent.
Basophiles	large	23	"
	small	13	"
Oxyphiles		2	"

No blasts seen. Much achromia.

February 9. Hemoglobin, 45 per cent. Reds, 4,096,000. Whites, 6,000.

Polymorphonuclear neutrophiles		76.0	per cent.
Basophiles	large	8.5	"
	small	15.0	"
Oxyphiles		.5	"

February 28. Hemoglobin, 35 per cent. Reds, 2,496,000. Whites, 9,300.

Polymorphonuclear neutrophiles		87.5	per cent.
Basophiles	large	1.0	“
	small	20.0	“
Oxyphiles		.5	“

No blasts seen. Some achromia.

March 18. Hemoglobin, 20 per cent. Reds, 1,944,000. Whites, 3,600.

Polymorphonuclear neutrophiles		67.3	per cent.
Basophiles	large	8.5	“
	small	23 7	“
Oxyphiles		.5	“

AUTOPSY. — Anatomical diagnosis. Pseudo-leukemia of retroperitoneal lymph glands, spleen and liver; general anemia; chronic hyperplasia of the spleen; fibroid atrophy of the narrow of the vertebra; hyperplasia of the marrow shaft of the femur; focal tuberculosis of the lung.

Skin pale. Edema of the ankles. Considerable fluid in the peritoneal cavity. One thousand centimeters of clear fluid in each pleural cavity. Left lung bound to parietal pleura by firm adhesions. Few adhesions at right apex. *Right Lung:* Near the apex a resistant area two by four centimeters, on section consisting of dense, fibrous bands separating semi-calcified areas. Similar area near apex of left lung, which on section consisted of grayish translucent nodules separated by fibrous tissue. In the center of this was a cheesy mass four millimeters in diameter. In the inferior lobe were similar areas four centimeters in diameter. *Heart:* Excess of serous fluid in pericardial sac. *Liver:* Not remarkable. *Spleen:* Weight three hundred and eighty-one grams. On section it was generally of a reddish-brown color, but scattered through it were several grayish-white, translucent areas. *Kidneys*, *Pelvic*, *Viscera*, and *Intestines* not remarkable. *Stomach:* Contained several small, soft polypi. In the retroperitoneal region on each side and in front of the aorta was an irregular tumor mass roughly sixteen by seven centimeters in diameter. On section this consisted of masses of irregular, ovoid tumors of a grayish, translucent, firm tissue. Attached to the pancreas was an ovoid tumor two centimeters in diameter, resembling a lymph gland on section. In the bodies of the lumbar vertebræ the fat was in excess of the red bone marrow. The shaft of the femur contained dark red marrow.

Microscopic Examination. — Sections from different parts of the tumor showed various stages, from a simple hyperplasia of the follicle to a dense gland formed mainly of fibrous tissue. A section of the retroperitoneal gland showed marked increase in the reticulum. No follicles could be distinguished. The blood vessels were fairly numerous and many showed endo-arteritis. The cells were mainly lymphoid. Epithelioid cells of varying size and shape with vesicular nuclei were common. A rare giant cell was seen. A marked feature of the specimen was the number of eosinophiles present. A section from a gland near the pancreas showed somewhat the same picture, only the fibrous tissue was considerably less. *Spleen:* Sections of the nodules showed them to be the same as the retroperitoneal tumor. Some of the follicles of the spleen were hyperplastic, while others were more or less transformed into fibrous tissue. Eosinophilic leucocytes were present in all parts of the spleen. *Liver:* The connective tissue about the bile ducts was increased and contained many small, round cells, eosinophiles, and a few cells of the epithelioid type. *Lung:* Showed well marked typical tubercular lesions of essentially the chronic type. *Marrow:* (*a*) Of femur showed extensive replacement of the fat tissue by marrow cells in most places, but in one part of the section it was not replaced, but necrotic. Among the marrow cells were many eosinophilic leucocytes. No megaloblasts were definitely recognized. (*b*) Of vertebra — the marrow spaces were largely filled with connective tissue, which in places contained marrow cells. The general impression was that the fat and marrow were extensively replaced by connective tissue.

No inoculation experiment was made.

CASE IV. — Dr. S. J. Mixter. Female, 55, widow. June 18, 1902.

Family and previous history not remarkable. Present illness, has been weak and lost considerable weight and strength in the last few months, but has never had any definite symptoms until about seven weeks ago, when she had an attack of indigestion characterized by pain in the epigastrium, occurring usually after meals, accompanied by nausea and vomiting. The vomitus consisted of large quantities of sour-tasting material and never contained any blood. Jaundiced since beginning of the attack. Six weeks ago had an attack of sharp pain in the right side of the chest and back, relieved by local treatment. Appetite poor. Constipated.

Physical Examination. — Well-developed and nourished. Skin and conjunctivæ yellow. A discrete, freely movable gland in the right posterior cervical triangle just above the clavicle the size of a hen's egg. Several other small ones in left. None in axillæ. Few in groins. *Chest:* Hyper-resonant on percussion. A few moist rales heard in the back. At the right apex voice sounds and resonance increased and a few fine rales heard on coughing. *Heart:* Apex in sixth space 4.5 inches from the sternum. Sounds faint. Soft systolic murmur at the base not transmitted. *Abdomen:* Liver two centimeters below the costal border. Apex of spleen palpable. Slight tenderness in epigastrium.

Urine showed bile and hyperemia.

The temperature varied from 98.6 to 102° C. Pulse and respiration not remarkable.

An exploratory laparotomy was done on June 27. The patient died three days later.

BLOOD. — Hemoglobin, 55 per cent. White count, 66,000.

Polymorphonuclear neutrophiles		85 per cent.
Basophiles	large	11 "
	small	4 "
Oxyphiles		1 "

AUTOPSY. — Anatomical diagnosis. Pseudo-leukemia of retroperitoneal, omental, supraclavicular, and pericardial lymph nodes and of the spleen; broncho-pneumonia; amalaceous secretions in the lung; icterus; accessory spleen; polyp of uterus.

Skin and conjunctivæ yellow. Moderate swelling in left supraclavicular region; two hundred to three hundred cubic centimeters of fluid in the pelvis. Large amount of peritoneal and omental fat, in which were many ill-defined areas of grayish, fairly firm tissue, the largest a few millimeters in diameter. In the greater omentum, near the stomach, were several rounded lymph-node-like nodules, the largest three centimeters in diameter, which consisted of soft, gray, homogeneous tissue. These had a definite capsule and resembled lymph nodes. The anterior surface of the lesser omentum showed many flattened nodules of the same tissue, and on section through these a conglomerate mass of numerous oval nodules of rather whitish, soft homogeneous tissue was disclosed. These varied up to two centimeters in diameter. The mass was connected with a second, lying about the pancreas and aorta, which was composed of larger nodules, varying up to seven centimeters in diameter, and which was situated, in general, between the pancreas and liver, but was continued downward along each side of the aorta, and occupied the situation of the postperitoneal lymph glands. All had a similar appearance to the nodules in the omentum. They had definite fine capsules and were loosely bound together. The mesentery near its root was invaded with similar nodules. *Spleen*: Weight, seven hundred and sixty-two grams. On section it was thickly sown with similar gray nodules varying up to four centimeters in diameter. Some were yellow, suggesting necrosis. *Liver:* Normal. Other abdominal and pelvic viscera not remarkable. *Thorax*: Left lung was bound to pleura by adhesions. In inferior lobes of both were two or three ovoid tumors similar to those elsewhere. No mediastinal or bronchial lymph glands. *Heart*; Normal. In the left supraclavicular region was a group of tumor nodules similar to the others.

Microscopic Examination. — Sections of the glands from various places showed about the same condition. All were very cellular and contained comparatively little fibrous tissue which, however, was more marked in some than in others. A section from a retroperitoneal gland showed it to be composed mostly of large epithelioid cells of varying shape and size, with large vesicular nuclei. The protoplasm often contained vacuoles. A few vessels could be seen, and the section showed necrotic areas, in many places infiltrated with polynuclear leucocytes. Many giant cells were present. Lymphoid cells were scattered through the section in small groups. (Plate XVI., Fig. 1.) *Spleen:* Showed a large amount of the same tissue which was extensively necrotic. Where not invaded it showed a marked increase in fibrous tissue. *Liver* and *Kidneys* not remarkable. *Lung*: Showed, in places, the air cells filled with a pneumonic exudate and presented the typical picture of a bronchial pneumonia. The marrow of the shaft of the femur showed the fat tissue much infiltrated with marrow cells.

Two guinea-pigs were inoculated subcutaneously on June 27. One with a small piece of the spleen, and the other with a bit of the growth. They were killed October 2. The autopsies failed to show any signs of tuberculosis.

CASE V. — Dr. S. J. Mixter. Female, five years. June 26, 1902.

Child has always been weak, often suffering from colds and diarrhea. At present has otitis media. For one year the parents have noticed a growing swelling on the left side of the neck. General condition the same as one year ago.

Physical Examination. — Poorly nourished. Examination of the chest and abdomen failed to show anything abnormal. On the left side of the neck, anterior to the mid point of the sterno-mastoid, is a discrete, oval swelling the size of a hen's egg, — not tender and freely movable. There are many small, discrete glands in the posterior triangle on the left and anterior triangle on the right. No glands in axillæ or groins.

Temperature, pulse, respiration, and urine not remarkable.

Pathological Examination. — Specimens consisted of several roughly oval, discrete nodules, varying from three to six centimeters in their greatest diameter. They showed a distinct capsule, and were loosely bound together by fibrous tissue. On section they were soft, homogeneous, of a yellowish-gray color. A few ill-defined fibrous trabeculæ seen near the capsule.

Microscopic examination showed no markings of the normal lymph glands. The sinuses were filled with cells. There was considerable increase in the reticulum. The cells, for the most part, were small lymphoid cells, but others with vesicular nuclei and a varying amount of protoplasm were abundant, being more common in some parts of the specimen than in others. Some were regular in shape, while others showed protoplasmic processes, and were intimately connected with the reticulum Blood vessels were fairly numerous and showed some endo-arteritis. A few eosinophiles were seen on search.

No inoculation experiments were made.

CASE VI. — Dr. H. F. Vickery and Dr. F. B. Harrington. Female, 54, widow. July 21, 1902.

Rheumatism ten years ago. Otherwise always well. Present illness: Grippe two years and a half ago, which was followed by a persistent cough with considerable sputum. Nineteen months ago she noticed a swelling under the left ear, soon followed by a similar one on the right side of the neck, which became the size of a goose egg in one month. At the same time smaller ones appeared above the clavicles, and she began to lose weight and strength. Three months later the swellings decreased in size and disappeared (?) in six months, while similar ones appeared in the axillæ and groins. For about a year she has had pruritis and night sweats, and has noticed a brownish pigmentation of the skin.

Physical Examination. — Well-developed, poorly nourished. Body perspiring and excoriated. Skin thickened and brown. Some edema of the ankles. In the triangles of the neck on both sides, and in the axillæ and groins are masses of hard, discrete glands varying up to four centimeters in diameter. *Heart:* Soft, systolic murmur, not transmitted, heard at apex and base. *Lungs:* normal. *Abdomen:* Spleen not enlarged. In the lower part a vague, irregular mass could be felt.

Urine, pulse, and respiration normal.

The temperature varied between normal and 100.

Blood. — Hemoglobin, 65 per cent. White count, 25,800.

Polymorphonuclear neutrophiles		81 per cent.
Basophiles,	large	13 "
	small	5 "
Oxyphiles		1 "

Some glands were removed from the groins August 8. Discharged unimproved August 31.

Pathological examination of a gland removed from the axilla, April 2, 1902, showed a firm, grayish nodule, two centimeters in diameter, invested with a tough capsule. On section it was composed of small areas of grayish, homogeneous tissue separated by dense fibrous septa.

Microscopic Examination. — The specimen consisted mainly of dense bands of connective tissue which separated small cellular areas. These were composed almost wholly of lymphoid cells. A few irregular, large cells with vesicular nuclei could be seen, which seemed to be connected with the reticulum. A few degenerated cells were seen, but no giant cells. There were very few vessels. (Plate XV., Fig. 2.)

The mass from the groin removed August 8 consisted of a number of rather soft, ovoid glands, varying from two to six centimeters in diameter. They were invested with a thin, fibrous capsule and loosely bound together. On section they were bulging and composed of fairly firm, homogeneous grayish tissue.

Microscopic examination of a gland showed what might be termed the cellular type of the disease. There was no evidence of any follicles. The reticulum was increased and present in fairly uniform amounts. Of the cellular elements the lymphoid cells predominated. There were, however, great numbers of the larger cells, with vesicular nuclei and protoplasm of various amounts. These cells were both free and connected with the reticulum, and many showed mitotic figures. Giant cells were abundant, and eosinophiles fairly common. The capsule was thickened and infiltrated with large cells and lymphocytes.

A pig was inoculated August 8 with a piece of a gland and killed October 2. The autopsy revealed nothing abnormal.

CASE VII. — Dr. F. B. Harrington. Male, 39, married. Sept. 2, 1902. Family and previous history not remarkable. Three weeks ago he noticed a bunch on the right side of neck which has increased rapidly in size until the present time. For the last three days he has had some dyspnea and difficulty in swallowing.

Physical Examination. — Well-developed and nourished. Throat negative. In the posterior cervical triangle, on the right, is a large mass of discrete glands, the largest of which is twelve centimeters, roughly, in diameter. A few smaller ones are present in the corresponding triangle on the left. None in axillæ. A few small, hard glands in both groins. *Chest:* Heart normal. *Lungs;* expiration slightly prolonged at right apex, otherwise normal. *Abdomen:* Spleen and liver of normal size.

Urine, temperature, pulse, and respiration not remarkable during his six days' stay at the hospital. The glands during this time decreased notably in size.

On Jan. 19, 1903, the glands in the neck were about the same as at discharge. Several discrete glands were present in the right axilla, the largest, roughly, four centimeters in diameter. A few smaller ones could be felt in the left axilla and in the groins. *Chest:* Normal. *Spleen:* Not enlarged. On April 15 the glands in the neck had increased somewhat in size, but, the patient said, varied considerably. The superficial veins over the right pectoral muscle were considerably dilated. On percussion to the right of the upper half of the sternum anteriorally was an indefinite area of diminished resonance, over which the breathing was somewhat harsh. The general condition was excellent.

Pathological Examination. — The specimen consisted of a few discrete lymph nodes from the neck, varying up to two centimeters in their greatest diameter. On section they appeared to bulge and were invested with fine fibrous capsules. They were homogeneous, and of a translucent grayish-yellow color. In some of the glands a few fine fibrous trabeculæ were seen dividing them into lobules.

Microscopic Examination. — Presented a picture almost identical to that described in the two preceding cases. There was no structure of the normal lymph gland to be made out. Lymphoid cells with the commonest cellular elements, but large, irregular, endothelial cells, were present in great numbers. Giant cells were also frequently seen, and there was a considerable number of eosinophiles. Plasma cells were present in larger numbers than in any other specimen, but were not a striking feature. Blood vessels were numerous.

Inoculation. — A pig was inoculated September 2 subcutaneously with a bit of a gland, and killed October 25. The autopsy showed the glands in the mediastinal axillæ, groins, and post-peritoneal considerably enlarged.

Microscopic examination of these glands showed simple hyperplasia and no evidence of tuberculosis, nor could the bacilli be demonstrated in sections especially stained for them.

CASE VIII. — Dr. C. B. Porter, Dr. F. C. Shattuck, Dr. E. G. Cutler. Female, 26, single. Nov. 21, 1902.

In October, 1899, noticed a swelling in the left side of her neck. Was examined at this time in the Out-Patient Department, and dullness and a few fine rales were found at right apex. In January, 1900, was sent to the Rutland Sanitorium for tuberculosis. While there had night sweats, but no tubercle bacilli were ever demonstrated in sputum. In August, 1901, a mass of glands was removed from the right side of the neck and right axilla at another hospital, but they recurred at once. They were removed again in May, 1902, but no pathological examination was made. At this time tuberculin was given with marked reaction. Three months ago the glands recurred in the left neck and axilla, and others appeared in the right side of the neck and axilla. Considerable cough and sputum for three years. Pruritis for last three months.

Physical Examination. — Fairly well-developed and nourished. In the left posterior cervical triangle are many small, firm, discrete glands not tender and freely movable. There is a similar mass on the right side, extending from behind the ear to the clavicle, and a freely movable rounded mass the size of a hen's egg below the right clavicle, and one a short distance below the left breast. A large mass of glands similar to those in the neck is to be felt in the left axilla, and a few in the right. There are also a few small glands in both groins. *Chest:* Heart normal. *Lungs:* Dullness and fine rales at the right apex. *Liver:* Normal. *Spleen:* Not enlarged. An ill-defined-resisting area can be felt above and to the right of the umbilicus.

On December 3 the glands on both sides of the neck and those of the right axilla were removed. On December 18 one thousand cubic centimeters of clear fluid was withdrawn from the right pleural cavity. This returned and approximately the same amount was withdrawn on January 2 and 18, March 7, and April 1. February 3 she was suffering considerable dyspnea and cough, and the glands had recurred on both sides of the neck. There was also an indefinite area of dullness under the sternum at the base of the heart, and an indefinite, slightly tender mass could be felt in the right iliac fossa. She died suddenly April 6, 1903, with dyspnea, cyanosis, and some exophthalmia.

No autopsy was allowed.

The temperature was very irregular, between normal and 103.

Pulse, respiration, and urine normal.

BLOOD. — Nov. 24, 1902.

Polymorphonuclear neutrophiles	80 per cent.
Basophiles	14 "
Oxyphiles	1 "

February, 1903. Hemoglobin, 85 per cent. Reds, 4,840,000. Whites, 16,000.

Polymorphonuclear neutrophiles	86.5 per cent.
Basophiles	12.5 "
Oxyphiles	1.0 "

Pathological examination of the glands removed from the neck. — The specimen consisted of a mass of lymph glands, in the aggregate about the size of the fist, with definite capsules and loosely bound together with fibrous tissue. On section, they were firm, bulging, of a grayish color, and traversed in places by fine fibrous trabeculæ.

Microscopic examination of a gland one centimeter in diameter showed no markings of a normal lymph gland. The reticulum was considerably increased, and the lymph spaces were filled with cells. Lymphoid cells of the larger and smaller forms constituted about one-half of those present, the greater part of the others having large, irregular, vesicular nuclei, and a small amount of protoplasm. These epithelioid cells were present in great numbers everywhere in the specimen, and many giant cells were seen, some with large and others with much smaller nuclei. There were many vessels. Eosinophiles were present in the tissue and in the vessels. Section from a gland eight centimeters in diameter presented the same appearance as the other, with the exception that a larger number of eosinophiles were present both in the vessels and in the tissue.

Inoculation. — Two guinea-pigs and a rabbit were inoculated December 3 with portions of a gland. One pig died the next day of an infection with gas bacillus. The rabbit died February 15. The autopsy revealed broncho-pneumonia, but no evidence of tuberculosis could be found microscopically. The second pig was killed March 2, 1903, but the autopsy showed nothing. A pig inoculated February 4 with some of the pleural exudate was killed April 9, and the autopsy revealed no signs of tuberculosis.

CASE IX. — Dr. C. L. Scudder. Male, 18, single. Feb. 10, 1903.

Children's diseases, otherwise always well. Six months ago noticed a swelling over the cartilage of the second rib on the left, which has gradually increased in size to the present time. No pain or tenderness, and no constitutional symptoms.

Physical Examination. — Well-developed and nourished. No glands in neck, axillæ, or groins. *Heart* and *Lungs* normal with the exception of an area of diminished resonance anteriorally over the base of the heart extending up to the sternum and one-half inch to the right to 2.5 inches to the left of that bone An indefinite mass could be felt at the sternal notch. Over the cartilage of the second rib was a soft tumor about two inches in diameter, not tender or painful. *Spleen* : Not enlarged.

Urine, pulse, respiration, and temperature normal the only time the patient was seen.

BLOOD. — Hemoglobin, 70 per cent. Red, 4,720,000. White, 23,000.

Polymorphonuclear neutrophiles		84.4 per cent.
Basophiles	large	5.0 "
	small	7.0 "
Oxyphiles		2.0 "
Mast cells		1.0 "

An incision was made into the tumor over the sternum and its contents curetted out.

Pathological Examination. — The specimen consisted of several small, irregular bits of yellow, necrotic-looking material and muscle with which the growth appeared to be infiltrating.

Microscopic Examination. — The specimen was for the greater part more or less necrotic, but in the better preserved places the reticulum was increased. The most striking thing was the number of eosinophilic leucocytes present which, in many fields, constituted about one-half the cells. The others were lymphocytes and large cells with irregular vesicular nuclei. Many giant cells with vesicular nuclei irregularly placed were also present. Many forms of degenerated cells were seen, and most of the fields showed a few leucocytes. Vessels were fairly abundant. (Plate XVI., Fig. 2.)

A guinea-pig was inoculated subcutaneously with a bit of the tumor on February 10, and died of unknown cause February 26. An autopsy showed an abscess at the seat of inoculation and hyperplastic glands in both groins. No tubercle bacilli could be demonstrated microscopically.

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DESCRIPTION OF PLATES.

The plates are photographs of drawings. The drawings were made by Miss Florence Byrns, the photographs by Mr. L. S. Brown.

PLATE XV.

Fig. 1. — Section of gland from the neck of Case II., showing the cellular stage of the disease. The cells present are the lymphoid and the large epithelioid type. The reticulum is increased. X 550.

Fig. 2. — Section of the gland from the axilla, Case VI., showing the fibrous stage of the disease. Small cellular islands separated by fibrous tissue. X 40.

PLATE XVI.

Fig. 1. — Section from a post-peritoneal gland of Case IV., showing the types of epithelioid cells. X 550.

Fig. 2. — Section from Case IX., showing the marked infiltration with eosinophilic leucocytes. X 550.

OBSERVATIONS ON THE DISTRIBUTION AND CULTURE OF THE CHANCROID BACILLUS.

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Ducrey[1] in 1889 first recognized and accurately described a bacillus which he found with great frequency in the pus of soft chancres. He failed to cultivate the organism on artificial media, but succeeded in obtaining it in a pure state in typical lesions in man, by successive inoculations of the pus. On this ground he based his belief that it was the specific cause of chancroid.

Soon afterwards these results were confirmed by Krefting,[2] who furthermore found the organism in the pus of buboes.

Unna,[3] a little later, demonstrated in sections of the tissue of soft chancres a similar bacillus occurring in long chains which he found constantly in the lesions, and which he called the strepto-bacillus of soft chancre.

Further investigations by Petersen,[4] Nicolle,[5] Cheinisse,[6] and others established the identity of the organisms of Ducrey and Unna. At the present time there is general agreement among bacteriologists upon the following points: Bacilli about 1.5 μ in length and 0.5 μ in thickness, with rounded, deep-staining ends, and a fainter staining central portion, occur with great constancy in the pus of chancroidal ulcerations, and less frequently in the pus of chancroidal buboes. These bacilli are found both singly and in masses, usually outside the pus cells, though sometimes within them. They occur also in very characteristic chains. In the tissues of excised chancroids the organisms are usually solid staining, and the arrangement in chains is particularly constant. They take basic aniline dyes well, but are quickly decolorized by alcohol, also by Gram's method of staining. Such bacilli have been observed hitherto in no other form of ulceration.

All attempts at cultivation of the organism prior to 1897 failed, though Petersen obtained non-characteristic growth in the depths of serum agar in one case, which failed to reproduce the disease when inoculated into man.

In 1897 Istamanoff and Akspianz[7] reported cultures in a medium of human skin and agar. The following year Lenglet[8] presented pure cultures of the bacillus of Ducrey before the French Society of Dermatology and Syphilography. The medium used was rather vaguely described as consisting of the proteid elements of human skin subjected to the action of certain energetic soluble ferments.

Inoculation of the pure culture into man reproduced the lesions, from which in turn new pure cultures were obtained.

Sections made of the artificially reproduced lesions showed chains of the bacilli in the tissues. Upon his special media the organisms grew out in long chains, the individual elements of which corresponded exactly in morphology and staining reaction to the well-recognized bacilli of Ducrey seen in the pus and tissues. No growth could be obtained in ordinary culture media. All attempts to reproduce the disease in animals failed.

In January, 1901, the convincing work of Bezançon, Griffon, and Le Sourd[9] upon the cultivation of the bacillus of Ducrey was published. These observers obtained pure cultures in four cases, two from chancroidal buboes opened under aseptic conditions, and two from extra-genital chancroids. Of the latter, one was the result of auto-inoculation from a genital lesion upon the thigh; the other was an artificial inoculation from a genital lesion upon the abdomen. The culture medium used was rabbit's blood agar in a proportion of one-third blood to two-thirds agar; also uncoagulated rabbit's blood serum. The colonies were described as appearing at the end of twenty-four hours, on the solid media, as bright, round globules which attained their complete development in forty-eight hours, becoming then opaque and grayish. They were one to two millimeters in diameter, and never became confluent. When stained and examined they were found to be composed of bacilli grouped in masses or arranged in short chains of three or four elements. In morphology these bacilli corresponded to the descriptions of the organisms of Ducrey and Unna. In the water of condensation the bacilli were found arranged in long sinuous chains in which the individual elements were smaller than in the surface colonies.

The growth of the bacilli was equally good in rabbit's blood serum uncoagulated. Their vitality in this medium, however, was short, while on the blood agar it exceeded three weeks. All attempts at cultivation on ordinary media failed, even after acclimation through a series of tubes of blood agar. Typical chancroidal lesions were reproduced in man by inoculation from pure cultures on three occasions. From these chancroids of inoculation the organism was recovered in pure culture. Animal inoculations were in all cases negative. Monkeys were not used.

This work of Bezançon and others has since been confirmed by Simon,[10] and very recently by Tomaszewski[17] in a small number of cases.

Nicolle[11] was the first to produce the disease in animals by inoculation of chancroidal pus. He succeeded in doing this in the year 1900 with certain species of monkeys, notably the Semnopithecus. More recently Holub, a Russian observer, has reported growth of the strepto-bacillus of Ducrey in the internal organs of various insects inoculated with chancroidal pus.

My own observation on chancroid, begun before the publication of the work of Bezançon and his co-laborers, and carried on for some time in ignorance of it, while confirmatory as to the prevalence of the Ducrey bacillus in chancroidal secretions, were negative as regards its cultivation, until media containing a considerable proportion of fresh blood, as recommended by the French observers, were employed. After a few initial failures with such media, pure cultures have latterly been obtained in eight cases.

The material made use of in these observations was available through the courtesy of the staff of the Surgical

Out-Patient Department of the Massachusetts General Hospital. One or two cases were from outside sources.*

An examination of the pus of forty cases of genital ulcerations clinically resembling chancroid was made. In thirty-two, bacilli were found corresponding exactly in morphology and staining reaction to the Ducrey bacillus — the characteristic chain arrangement occurring in ten of these. In eight cases it was impossible to positively identify the Ducrey bacillus in the varied assortment of bacteria present. As improvement in technique in taking smears and increasing familiarity with the bacillus were gained, the percentage of these negative cases diminished. The bacilli were found as the prevailing organisms in the simple ulcers, as well as in the phagedenic destructive ones, and in equal numbers proportionately; seeming to show that the virulence of the process depends largely upon the resistance of the individual, an hypothesis in accord with clinical observation. Chain arrangement was noted only in cases which were actively progressing, while the location within the cells was particularly noticeable in those which were healing.

In one case, which resembled chancroid clinically, and in which Ducrey bacilli were found, secondary symptoms of syphilis developed later; this was the only occurrence noted of mixed infection.

Sections of six genital chancroids which were excised showed Ducrey bacilli in only one. The sections were stained with Loeffler's methylene blue or Unna's blue, dehydrated and cleared in aniline oil and xylol. The difficulty experienced in dehydration and clearing without decolorization of the organisms largely explains the negative results.

Pure cultures of the Ducrey bacillus were obtained directly from the pus of primary genital chancroids in two cases. In many others there was growth of the organism in culture, which could not, however, be isolated from various contaminating bacteria. One instance of chancroidal ulcerations on the skin of the abdomen, resulting from accidental auto-inoculation from a genital lesion, also yielded pure cultures. Pure cultures were likewise obtained from chancroidal buboes opened under aseptic conditions in two cases, and from the pus of a chronic discharging bubo in one case. In four other cases of chancroidal buboes, in which the same media were used, there was no growth of any kind. Of these seven buboes, in only two were Ducrey bacilli found in smear preparations of the pus. In two cases (I. and III.) of extra-genital chancroids of the fingers, which I believe to be unique, in that there were no genital lesions, pure cultures of the same organism were obtained, — in one from the ulcer direct, in the other from the pus of an abscess secondary to ulcerations on the finger.

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* The photograph of the extra-genital chancroid is reproduced through the kindness of Dr. E. A. Codman, in whose service at the Massachusetts General Hospital the case was treated. For valuable advice and assistance in carrying on this work I wish to express my deep obligation to Dr. J. H. Wright.

The cultures from these eight cases, which were without contamination from the start, were identical in every detail observed, and coincided accurately with the description of Lenglet, and Bezançon, and others.

The medium used at first was rabbit's blood agar. Tubes of two per cent agar, reaction + 1.5, were melted and mixed with fresh rabbit's blood, drawn under aseptic conditions, in the proportion of two-thirds agar to one-third blood, and slanted while in a fluid state. At a later period tubes of rabbit's blood serum uncoagulated, also rabbit's blood bouillon, one-third blood to two-thirds bouillon, were used, and gave equally satisfactory results.

By employing small tubes of freshly drawn human blood, pure cultures were obtained in several instances from genital lesions direct, without any special cleansing of the ulcerated surface. This, I believe, is the best medium for obtaining cultures from a source open to contamination, the fresh blood apparently inhibiting to a certain extent the growth of extraneous organisms.

The method of preparing the media was as follows: A needle prick is made on the extensor surface of the thumb just back of the base of the nail, the skin having been previously cleansed with soap and water and alcohol. By applying a tourniquet at base of thumb just tight enough to obstruct the venous flow, a considerable quantity of blood may be obtained. The blood is drawn up by capillary attraction into a small glass tube, one end of which has been drawn out to a very fine caliber over the Bunsen flame, and the whole tube sterilized by the same means. When about two-tenths of a cubic centimeter of blood has flowed into the tube, its capillary end may be sealed in the flame and the other end plugged with cotton, giving a miniature culture tube. The media were frequently tested after having been twenty-four hours in the thermostat at 37° C. and found sterile in all instances. All these blood media deteriorate with age, and after two or three weeks, although sterile, they are found lacking in nutrient qualities for the Ducrey bacillus.

The first manifestation of growth of the organism, after twenty-four hours in the thermostat at 37° C., was a formation of delicate flocculi in the fluid media, or in the water of condensation of the blood agar tubes. Examination of this fluid in cover-glass preparations, under the microscope, showed many chain groups made up of individual bacilli, corresponding in morphology and staining reaction to those observed in the purulent secretions. Isolated bacilli were very rarely seen. The chains in some cases were of extreme length, extending through several fields of the microscope, often in parallel lines, usually tortuous, less frequently straight, but most often tangled in the greatest confusion.

In all cultures a few long, undivided filaments were observed, often attaining a length of ten to twenty times, or more, greater than that of the individual bacillus. (Such forms were described by Lenglet also.) Transitional forms, intermediate in length and morphology between typical bacilli and the above-mentioned filaments, retaining the deeply stained rounded ends and arrangement in chains, were also observed and are shown (Fig. 13). These filamentous forms were found in both twenty-four-hour and forty-eight-hour cultures, but were far commoner in the latter, and I think are to be regarded as degenerative forms. No branching was at any time observed either in the chains or filaments.

After flooding the surface of the solid blood agar media with the water of condensation, containing a twenty-four-hour growth, small, round, grayish colonies were observed at the end of forty-eight hours. These colonies were difficult to pick up on the platinum needle, as they tended to glide in front of it. When examined under the microscope they were found to consist of the same organisms arranged in short chains and singly. The bacilli of the colonies quickly underwent involution forms, becoming misshaped and swollen and taking the stain irregularly. The same phenomena were noted in the fluid media in forty-eight to seventy-two hours. The most satisfactory stain for the bacillus in culture, as well as in the pus, was found to be carbol fuchsin followed by brief decolorization with alcohol, which brings out the bi-polar staining well. The bacilli decolorized by Gram's method as well as by mineral acids. No spore formation was observed. In hanging-drop preparations no motility could be made out. The vitality of the cultures was short, rarely exceeding three days, and in no case four days. In one case cultivation without contamination was carried to the eleventh generation with some diminution of luxuriance of growth, but without change in morphology; further cultivation was not attempted. Subcultures upon ordinary media repeatedly attempted in all cases were invariably negative, the media tried being coagulated ox blood serum, uncoagulated ox blood serum filtered, potato, agar, glucose agar, glycerine agar, agar with surface smeared with blood, hydrocele agar, hydrocele fluid, gelatin, bouillon, and egg albumen. Anaerobic cultures (Wright's method) on ordinary media were also negative, while abundant growth occurred on fresh blood media anaerobic by the same method.

Inoculation of ordinary laboratory animals, which previous observers had found invariably negative in many trials, was attempted only once. A guinea-pig was inoculated by rubbing in two loopfuls of a pure twenty-four-hour culture upon a scarified area on the back. A dry scab formed, which dropped off in three days without causing reaction of any kind.

A monkey of the genus Macaccus Nemestrinus, inoculated by the same method on the back, from the same culture obtained from Case I. showed at the end of eight days considerable elevation of the crust which had formed at the seat of inoculation. Upon lifting off the crust, a shallow ulceration was disclosed about four millimeters in diameter, containing a drop or two of pus.

A smear preparation made of the pus showed a few isolated bacilli corresponding to the Ducrey bacillus in morphology, lying within and outside the pus cells. No chain arrangement was seen. There were also a few large bacilli and cocci present. Cultures on rabbit's blood bouillon and blood serum were made from this pus. There was no growth in the blood serum tube. In the rabbit blood bouillon tube there was a profuse growth of bi-polar staining bacilli arranged in long chains exactly corresponding in morphology and staining reaction to those of the parent culture (Fig. 9). There was also a slight contamination with a long filamentous organism of large diameter with square ends, which was afterwards isolated in the depths of glucose agar. Subcultures upon all other ordinary media were negative. At the end of fifteen days the ulceration on the monkey's back had entirely healed. There was neither glandular enlargement nor constitutional symptoms at any time. At a later period inoculation of the same animal on two occasions with chancroidal pus containing bacilli (Case V.) resulted at the end of twelve days in the production of shallow ulcerations containing a few drops of pus. In this pus Ducrey bacilli were found on several occasions, almost in a pure state, arranged often in short chains (Fig. 5). Cultures in rabbit's blood bouillon from one of these ulcerations gave a typical pure growth of the organism (Fig. 10), in the other there was contamination with cocci. The ulcerations on the monkey pursued a mild course, and were entirely healed without treatment in three weeks.

In view of the extra-genital source of two of my cultures, it may be of interest to briefly review some statistics on this subject.

Ullman [14] has recently (June, 1902) published a collection of cases of extra-genital chancroid taken from the literature. His list comprises only what he considers authentic cases. Of these there are sixty-four, of which twenty-two occurred upon the fingers. In all of these twenty-two, genital lesions also were present. Nine cases were verified by finding Ducrey bacilli in the pus, four by positive inoculation, none by culture. It is interesting to note that in one of these cases reported by E. Lang there were multiple abscesses in the course of the lymphatics on the back of the hand, as in Case III. of my series.

Ullman also quotes some figures of O. Peterson's, which are not included in his own tables. Peterson collected nine thousand cases of soft chancre, of which 8,992 were located on the genitalia, or the perigenital regions (buttocks, groins, etc.), leaving eight of extra-genital site, of which five were upon the fingers.

Queyrat,[15] out of fifteen hundred cases seen by him at the Ricord Hospital, mentions only five cases in which the hands were affected. In four of these Ducrey bacilli were found in the pus. Genital lesions existed in all.

CLINICAL HISTORIES OF CASES FROM WHICH PURE CULTURES OF THE DUCREY BACILLUS WERE OBTAINED.

CASE I. — Dec. 28, 1902. Young man about twenty-six years of age, of previous good health, came to me with following history: Five days previously had run a splinter of wood into middle finger of right hand close to the base of nail. He immediately extracted the splinter. Four days later the finger caused him some pain and interfered with sleep that night. Examination on the next day showed a localized area of moderate swelling, redness, and tenderness about base of nail. After applying sulpho-naphthol poultices for two days, it became apparent that there was pus present, and a small incision under cocaine anesthesia evacuated about two drops of pus. An antiseptic dressing was applied. The pain continued, however; there was a daily discharge of pus, exuberant granulations sprang up through the incision, and altogether the wound presented an unsatisfactory appearance, although there was no extension of the process up the finger.

January 8. Under primary ether anesthesia, a crucial incision was made, the exuberant granulations curetted out, and the wound thoroughly irrigated with corrosive sublimate 1-1000, and packed with gauze. This was followed by relief from pain, but the wound showed no tendency to heal; on the contrary the line of incision became ulcerated and undermined, and presented the appearance of a phagedenic ulcer with dirty grayish base, exuding yellow pus, surrounded by an area of moderate swelling without special induration. There was no visible extension along the lymphatics, but the epitrochlear gland was slightly enlarged. No axillary glands were to be felt at this time. The ulceration slowly extended during the next week, attaining the size of the thumb nail.

January 15. A smear preparation of the pus showed bacilli in large numbers indistinguishable from the Ducrey bacillus arranged as in Fig. 3, apparently without contamination. A culture on blood serum made at this time showing no growth, the diagnosis of probable extra-genital chancroid was made, although there was no genital lesion, nor had there been one previously. The wound was washed out with peroxide and corrosive twice daily, and also soaked in sulpho-naphthol at home.

For the next few days smears from the pus showed the same bacilli in large numbers. Cultures on agar agar smeared with blood and blood serum were tried repeatedly, but remained sterile in all cases.

January 17. Case seen in consultation with Dr. E. H. Nichols, who concurred in the diagnosis. The wound was now treated with crude carbolic and powdered iodoform; the process continued to extend nevertheless, burrowing under the skin, and forming a pocket below the ulceration, which contained four or five drops of pus.

January 25. Cultures made on rabbit's blood agar and ox blood serum eight hours after dressing. Guinea-pig inoculated subcutaneously with 1.5 cubic centimeters of a suspension of the pus in bouillon, there being two loopfuls of pus to three centimeters of the bouillon. Second pig inoculated with equal dose by intra-peritoneal injection. Some of the same pus planted on potato culture.

January 27. Culture tubes negative, except rabbit blood tube of January 25, which showed cloudiness in water of condensation. Examination of the water showed pure growth of typical Ducrey bacilli in chains. Sub-cultures were carried on for eleven generations from this tube until February 12.

Cultures of the same organism were also obtained from the ulceration on three subsequent occasions, January 28 and 30 and February 4, pure in the first two instances, but contaminated with cocci in the last.

The clinical appearance of the ulceration on the finger continued about the same until February 4, when after several applications of saturated permanganate of potash solution it began to clean up with diminution of the purulent discharge. Smear preparations of the pus after February 9 showed very scanty Ducrey bacilli, mostly within the leucocytes. The pus pocket, which had burrowed under the skin, closed spontaneously, healthy granulations appeared on all sides, and on February 14 the process had entirely healed. At this time the epitrochlear gland was still palpable, but not tender; small glands were also to be felt in the axilla, not tender.

The patient was last seen April 1, when he was still perfectly well, no sequelæ of any kind having developed. The etiology in this case remains obscure, although the possibility of venereal contact shortly after the injury to the finger, and three days before I saw him, is admitted by the patient.

The guinea-pig with intra-peritoneal inoculation of the pus appeared very dull and quiet for a couple of days, but afterwards seemed as well as ever. He was killed on the eighth day, but showed no lesions of any kind. The other pig showed no reaction at any time and was allowed to live. A monkey inoculated as above described with a pure twenty-four-hour culture of fifth generation from this case showed at the end of eight days a shallow ulcerative process, containing pus from which a culture of the original organism was recovered.

CASE II. — Feb. 7, 1903. P. S., 25 years, laborer. One month previous had sore on penis, which is now healed, leaving small scar back of corona. In the right groin there is a painful, reddened, and tender swelling of two weeks' duration. On February 11, the bubo being fluctuant, an incision was made under aseptic conditions, and about thirty cubic centimeters of pus were evacuated. Smear preparations of this pus showed no bacteria of any kind. Cultures were made on ordinary blood serum and rabbit blood bouillon. No growth on the former, in the latter pure growth of Ducrey bacilli.

CASE III. — Feb. 14, 1903. M. R., 30, tailor. About one month previously had pricked middle finger of right hand with a needle. Shortly afterwards the finger became sore, and about a week later tender swellings appeared on the back of hand and at elbow. He was treated for a time at another hospital, where incisions were made in the finger, and also on the back of hand and at elbow.

Examination at Massachusetts General Hospital clinic showed a fairly developed and nourished man, presenting on the palmar aspect of the second phalanx of middle finger of right hand a ragged ulceration with undermined edges and dirty base, about size of five-cent piece, with very little surrounding inflammation and no induration. A similar ulceration existed on the lateral aspect of the same finger. On the dorsum of the hand was another ulceration, and just above this a moderately tender fluctuating swelling. In the epitrochlear region, along the line of the brachial vessels, were four similar swellings, one of which had been opened and was discharging pus. There was no apparent lymphangitis in forearm and no tenderness or swelling. General condition excellent, temperature and pulse normal. There was no venereal disease present, and venereal contact of any kind was denied.

Small abscess on back of hand was opened under aseptic precautions. Smear preparations of the pus showed a few scattered Gram decolorizing bacilli similar to Ducrey bacilli in morphology. (Fig. 4.)

Cultures were taken on ordinary blood serum and in rabbit blood bouillon. There was no growth in the former, but a profuse growth of typical Ducrey bacilli in the latter medium. (Fig. 11.)

Under antiseptic treatment the ulcerations slowly healed, and the abscesses, after discharging profusely for a time, likewise closed up. The axillary glands became considerably enlarged and tender at one time, but later subsided without suppuration. When last seen at the end of March the patient was ready to return to work.

CASE IV. — March 5, 1903. G.A., 25 years. Four weeks ago first noticed sores on penis coming on two days after coitus. Two weeks later noticed similar sores on abdomen caused by scratching pimples. Examination showed several superficial non-indurated ulcers on glans and contiguous parts of prepuce. On the skin of right groin and right hypochondrium were two small, undermined ulcerations. A few Ducrey bacilli were found in smears from ulcerations on penis; from ulcerations on skin of abdominal wall they were found in great numbers, arranged in characteristic chains. The extra-genital ulcerations were washed with hydrogen peroxide, and corrosive sublimate 1–1000, and sealed under sterile collodion dressings. Cultures were taken from the pus, which had accumulated under the dressings after twenty-four hours, in two weeks' old rabbit blood bouillon. Growth of cocci and colon bacilli were obtained; no Ducrey. On March 13 a pure growth of the latter was obtained on freshly drawn rabbit's blood from one of the abdominal ulcerations which had been cauterized with crude carbolic acid, and sealed for twenty-four hours under an aseptic dressing.

CASE V. — Aug. 8, 1902. D. C., 29 years. Came to hospital with enlarged tender glands in right groin. Genital organs normal. On September 19 the glands broke down spontaneously. The resulting sinus was enlarged, and the wound dressed regularly at the hospital. On October 17 it was healed. On Feb. 14, 1903, the patient returned to the clinic with three small ulcerations on prepuce near frænum, which had appeared about five days before. The scar of bubo in right groin, which had been healed for four months, had broken down again, and there was a purulent discharge from the sinus. Smear preparations of the pus from preputial ulcers, also from discharging sinus in groin, showed numerous Ducrey bacilli. On February 28 monkey was inoculated on the skin of back with the purulent secretions from the sores on penis. On March 12 there was a well-developed ulceration on skin of monkey at seat of inoculation, from which Ducrey bacilli were recovered in pure culture as already described. (Fig. 10.)

On March 16 a pure culture of the Ducrey bacilli was obtained from the pus of the discharging bubo in rabbit's blood bouillon. (Fig. 7.)

At the end of March, when last seen, the chancroids were nearly healed; the bubo, however, was still discharging.

CASE VI. — March 9, 1903. J. H., 23 years. Doubtful history of syphilis one and a half years ago. Five weeks ago first noticed sore on penis; three weeks later a tender lump appeared in left groin.

Examination showed a phagedenic ulceration of the skin of shaft of penis, almost encircling the organ. In the left groin there was a mass of matted glands. Smear preparations of the pus from the chancroid showed abundant Ducrey bacilli in short chains. (Fig. 1.)

On March 15 the bubo was opened under ether, and considerable pus evacuated; the chancroid was curetted as well. No bacilli found in smears of the pus of bubo. No growth in rabbit's blood bouillon. Profuse growth of Ducrey bacilli obtained in the same media directly from chancroid. No growth on ordinary blood serum.

CASE VII. — M. C. Small, non-indurated ulceration on prepuce near frænum, enlarged tender glands in groin, short chains of Ducrey bacilli in smear preparations of pus from sore. On March 26 a pure growth of Ducrey bacilli were obtained in tubes of fresh human blood directly from the genital sore, without special attempt to disinfect its surface. Cultures on ordinary blood serum taken at the same time showed profuse growth of cocci.

CASE VIII. — March 27, 1903. P. A. Two weeks ago, four days after coitus, sore appeared on penis. A few days later noticed pain and swelling in right groin. Examination showed small, non-indurated ulceration just back of corona. Tender, reddened, fluctuating swelling in right groin. Smear of secretion from ulcer showed a few Gram decolorizing bacilli, resembling Ducrey in morphology, mixed with a large number of extraneous organisms. Bubo incised under aseptic conditions. A very few Ducrey bacilli found in the pus. Cultures made on blood serum and fresh human blood. No growth in former, in latter pure growth of Ducrey bacilli.

SUMMARY.

The results of this investigation contribute in some measure to the accumulating evidence of the specific agency of the bacillus of Ducrey in the causation of chancroid and its associated bubo; and I believe justify the following conclusions:

1. The organism is present in the purulent secretions of the great majority of cases of chancroid and occasionally also in the pus of buboes, and may be identified by its morphology and staining reaction, together with its inability to grow on ordinary culture media.

2. Characteristic growth of the organism in a pure state

may be obtained in suitable media from genital chancroids direct and also from chancroidal buboes in some cases. Growth is most luxuriant in a medium of fresh blood and bouillon, but unmixed human blood is the best medium for obtaining cultures from a source open to contamination.

3. Inoculation of a pure culture of the bacillus or of chancroidal pus upon the skin of a certain species of monkey reproduces the lesion, from which in turn the original organism may be recovered in culture.

4. The cultivation of the same organism in a pure state from lesions on the hands, clinically resembling chancroid, in the absence of genital lesions, indicates that chancroid may be primary upon an extra-genital site.

5. Bacteriological examination of all ulcers of this type is likely to establish in the future a greater prevalence of extragenital chancroid than has hitherto been reported.

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DESCRIPTION OF PLATES.

(*Photomicrographs by Mr. L. S. Brown.*)

Both smear and culture preparations were stained with carbol-fuchsin, and briefly decolorized by alcohol. Cultures were of twenty-four hours' growth in rabbit's blood bouillon, unless otherwise specified, and were without contamination, except in Fig. 9.

Magnification one thousand five hundred diameters.

PLATE XVII.

Fig. 1. Smear of pus of chancroid of penis, Case VI.

Fig. 2. Smear of pus of bubo, Case V.

Fig. 3. Smear of pus of chancroid of finger, Case I.

Fig. 4. Smear of pus of abscess secondary to chancroids of finger, Case III.

Fig. 5. Smear of pus of ulceration on monkey resulting from inoculation of pus of genital chancroid, Case V.

PLATE XVIII.

Fig. 6. Culture from chancroid of penis. Medium, fresh rabbit's blood, first generation, Case VI.

Fig. 7. Culture from chancroidal bubo, second generation, Case V.

Fig. 8. Culture from chancroid of finger, third generation, Case I.

Fig. 9. Culture from ulceration on monkey resulting from inoculation of culture from Case I., first generation.

Fig. 10. Culture from ulceration on monkey resulting from inoculation of pus from genital chancroid in Case V.

PLATE XIX.

Fig. 11. Culture from abscess secondary to chancroids of finger, Case III.

Fig. 12. Forty-eight-hour culture, first generation water of condensation in rabbit blood agar tube. Typical bacilli and filament, Case I.

Fig. 13. Same culture. Transitional forms.

Fig. 14. Primary ulceration on finger with secondary abscesses on back of hand and at elbow, Case III.

CHRONIC CYSTIC MASTITIS. — A STUDY OF THIRTY CASES.*

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The fibrous and cystic changes which occur in the female breast at about the time of the menopause have long been a matter of interest in surgery. Occurring at the time of life when malignant disease is most to be dreaded, and presenting a clinical picture which closely resembles that of cancer, these changes are of extreme importance from a diagnostic point of view. A greater significance attaches to them, however, in the minds of many surgeons because of the belief that in these involution changes occur the first steps of epithelial proliferation which ultimately lead to cancer.

Increase of the fibrous tissue and the frequent presence of cysts in the breasts of women past middle life are conditions which have been described by many writers and under many different names. Chronic Interstitial Mastitis of English writers, Chronic Cirrhosing Mastitis (Billroth[5]), Maladie Cystique de la Mammelle or Intra-acinous Cystic Epithelioma (Reclus[33]), Maladie de Reclus, Maladie Noueuse de la Mammelle (Phocas[29]), Chronic Cystic Mastitis (Koenig[20]), Periductal Mastitis (Delbet[15]), Poly-cystoma or Cyst-adenoma (Schimmelbusch[39]), Plexiform Fibroma (French writers), Cystic Disease of the Breast (Bryant[9]), and Fibrous Hyperplasia with Retention Cysts; all of these designations have been applied to this disease.

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* Of the thirty cases upon which this report is based, seventeen were treated in the wards of the Massachusetts General Hospital, and were under the care of the surgeons of the staff. The remainder of the cases, thirteen in number, were obtained from the private practice of the following surgeons of this city: Drs. C. B. Porter, J. C. Warren, A. T. Cabot, M. H. Richardson, C. L. Scudder, Anna G. Richardson, H. F. Vickery, G. W. W. Brewster, and Farrar Cobb. To all of these surgeons the writers would here express their gratitude for the permission to report the cases and for the clinical details of history and treatment. To Dr. W. F. Whitney and to Dr. J. H. Wright, of the Massachusetts General Hospital, the writers feel themselves under especial obligation, not only for the generous use which they have allowed of their material, but also for much active interest and suggestive criticism during the course of the investigation.

As may be deduced from the variety of names which have been given to this condition, different interpretations have been placed by different observers upon the pathological picture which it presents. In the main, this difference of interpretation has been influenced by the individual or even national belief of the observer, with regard to the line of division between inflammatory hyperplasia and tumor formation. In the breast, this problem presents especial difficulties, because of the intimate admixture of epithelial and connective tissue elements, and the participation of both of them in almost every pathological process, whether inflammatory or neoplastic. By the modern French school the lesions of chronic cystic mastitis are regarded as of inflammatory origin. By many German writers they are considered actual new growths and dignified by the name of Cystadenoma. Other writers take a position between these two extremes, and admit that the line of division is one which in certain cases can be drawn with difficulty or not at all. In England, especially, much has been written concerning the association between chronic mastitis and cancer. The evidence to support this relation has been derived for the most part from the examination of breasts removed for cancer which presented lesions in the breast tissue beyond the limits of the cancer growth, which were interpreted as chronic mastitis. These observations, together with the clinical evidence of the development of cancer in breasts which were previously attacked with chronic mastitis, have aroused a general distrust of the disease in England and in this country. To establish this distrust upon a more secure foundation is the object of this communication.

The technique employed in the study of the thirty cases here presented was as follows: Material from the operating rooms was examined in the fresh state, and sections were made of the entire specimen. Small pieces from many different portions of the specimen were then hardened in Zenker's fluid. After thorough washing the fixed specimens were put through ascending alcohols and chloroform and embedded in paraffin. Sections were cut with the Minot-Blake microtome, two and three micro-millimeters in thickness, and stained with iron-hematoxylon (Mallory) and orange G. and fuchsin. Serial sections were made of tissues of special interest, and a number of other methods of staining were employed in appropriate cases. Specimens from operations in private practice were occasionally available only after fixation in formalin (5 or 10 per cent), and such specimens were as a rule inferior to the Zenker hardened ones for histological purposes.

In the study of the disease of an organ a knowledge of its normal appearances is essential. For this reason a short summary of the normal histology of the breast may here be introduced.

Shortly after puberty the female breast presents a typical picture. In a framework of interlobular connective tissue, interspersed with fat, the main ducts and the smaller lobules of the breast are widely scattered. Around each duct, and especially around each acinus, is found the cellular and delicate "periductal" connective tissue, which is peculiar to the breast. The main ducts are empty and infolded and possess a double layer of small columnar epithelial cells. The acini are small and empty, and are lined with a single or double layer of small cuboidal cells. The blood vessels are small and few in number.

With increasing age there is a slight increase in the number of the lobules, but no marked change occurs in their constituents. During pregnancy active increase takes place, not only in the number of lobules, but in the number of acini in each lobule. The gland is grossly enlarged and new blood vessels appear. The epithelium of the acini increases in development, and evidence of active growth by mitosis has been observed (Steinhaus[43]). The products of secretion are seen in the cells and filling the lumen of the acini and ducts.

During lactation the vascularity of the gland is maintained as well as the high degree of developement of acini and epithelium. Secretion products of all stages are found in the acinal epithelium and in the lumen of the acini and ducts. The acinal epithelium rarely exceeds one layer of cells in thickness at this time.

After lactation a degree of involution occurs, and the large number of acini gradually diminish. A breast which has undergone lactation rarely returns, however, to its previous condition, and a larger number of acini than in the virgin breast can generally be demonstrated. With this diminution in the number and size of the lobules, an increase of the interlobular fibrous tissue occurs in the meshes of which the accumulation of fatty tissue is abundant.

At about the time of the menopause, between the ages of thirty-five and fifty, further involution changes take place as the functional activity of the gland is no longer required. This process occurs to the same extent as a rule in breasts which have undergone lactation and in those which have not. It consists in a further diminution in the number of glandular elements, even to the almost complete disappearance of the acini in some cases, and the formation of a dense interlobular fibrous tissue with few nuclei and with a heavy fibrillated structure. (Plate XX., Fig. 1.) The fatty tissue interspersed in the meshes of this interlobular fibrous tissue is variable in amount, and becomes gradually smaller in quantity as the fibrous tissue undergoes increase. The epithelium of the atrophic acini is small and irregular, and is disposed in one thin layer as a rule. The epithelium of the ducts is less affected, and is frequently arranged in a double layer. The peculiar periductal tissue of the breast either disappears or undergoes hyaline degeneration. The blood vessels are shrunken and few in number. This statement of the normal histology of the breast is based upon numerous specimens examined by the writers, and is controlled by the reports of similar observations by others. (Steinhaus,[43] Bizzozero and Ottolengui,[7] Warren.[46])

The thirty cases of this series presented the lesions of chronic cystic mastitis. In three the lesions of carcinoma were found in other portions of the breast, accompanying the lesions of chronic cystic mastitis. The consideration of these three cases will be deferred until after the results of the study of the simple cases have been recorded. Twenty-seven cases of uncomplicated chronic cystic mastitis presented the following clinical characteristics:

The age of the patient was stated in twenty-five cases. In sixteen cases the age was between forty and fifty years. The youngest case was twenty-five years old and the oldest fifty-two. The average being 40.4 years. This is in accordance with the view of most observers, although it shows the age incidence to be a trifle earlier than the age at which the menopause is commonly expected. The disease is therefore one which is more likely to precede the menopause than to follow it. Three cases occurred in women under thirty years of age, but the specimens in these cases presented no appreciable differences, either clinically or histologically, from those of older women.

The influence of child-birth and of lactation was not especially prominent in this series of cases. Nineteen women were married. Eight were single women. Ten of the married women had borne children; seven had borne no children, and in two cases the records were deficient. Thus, of twenty-five women suffering from this disease, ten had had children and fifteen had not. Whether or no lactation was prolonged or of brief duration, in the cases which had borne children, could not be ascertained. In a few cases the fact that lactation was present, but was suddenly interrupted, owing to the death of the child or to other considerations, has appeared to the writers to have some importance in the etiology of the disease.

An injury to the breast preceded the appearance of the trouble in only one case of the entire number. In another case infantile mastitis occurred in early life on both sides, and was followed by bilateral chronic cystic mastitis at the age of twenty-six. No case of puerperal breast abscess was noted in the entire number of cases.

Uterine disease and disorders of menstruation have been noted by several writers as accompanying this condition. Two of these cases had passed the menopause. Twelve gave a history of normal and regular menstruation, and only two cases complained of irregular and painful catamenia. When painful menstruation is present, however, it has been noted by the writers that exacerbation of the disease, with each recurring period, is even more marked than in the cases with normal menstruation. Koenig calls particular attention to this phenomenon, and attributes the increase of tenderness and even the actual increase in size of the indurated area which is occasionally noted in these cases to the congestion and tumefaction which normally occur in the breast at the time of the menstrual period.

The duration of the disease, before the patient sought medical advice, was a matter of considerable variation in this series of cases. The induration was, as a rule, discovered by the patient by chance, and a longer or shorter time elapsed between the discovery of the tumor and the seeking of medical advice, according to the state of intelligence of the patient, or the degree of anxiety which she evinced with regard to the presence of an indurated mass in her breast. Seven cases sought advice within one month after the discovery of the tumor; eight cases from one to six months after; and six cases at varying periods of time up to four years after the time when the tumor was first detected. In six cases the duration of the disease was not recorded. The shortest period elapsed between the discovery of the mass and the resort to medical advice was two days. It is evident that no deductions can be drawn from these data, with regard to the length of time the actual induration had existed.

The onset and growth of the disease in the breast was regarded as rapid in six cases and as slow in eleven cases. Those which presented a rapid increase of size were, as a rule, the cases in which a cyst of considerable size was present and the rapid increase in size could be attributed to the collection of fluid in the cyst.

Both breasts were involved in ten cases, and only one breast in eleven cases. In six cases the condition of the other breast was not recorded. When bilateral, the disease was as a rule more marked on one side than on the other.

An induration of the breast tissue was present in every case. This induration varied from a mass, which was regarded as a tumor in ten cases, to a degree of increased consistency of the gland which was with difficulty to be appreciated. It is important in the examination of these cases, as pointed out by several writers, that the breast should be examined under the flat of the hand, and that the gland should be pressed against the chest wall, instead of being pinched up between the fingers of the examiner. When pinched up in this way a double thickness of gland tissue is felt, and the impression is readily obtained of a tumor mass not justified by an examination under the flat of the fingers. The areas of increased consistency were rarely well defined at their margins, but faded gradually into the surrounding gland tissue. When cysts of any considerable size were present, the limits of the induration were more sharply marked. In some cases an irregular "corded" feeling of the gland, with minute, more indurated spots, was all that could be detected by physical examination.

The situation of the induration in the breast was of interest. In twelve cases the disease was spread diffusely throughout the breast. In two of this number, however, the part first and most affected was the outer hemisphere. In thirteen cases the disease was localized more particularly in one portion of the breast. Of this number, eight cases showed an involvement of the outer hemisphere, two of the lower hemisphere, and two of the central portion of the breast. As in cancer, the upper and inner quadrant was the part which was most rarely affected, and the upper and outer quadrant the one most liable to attack. Where the whole breast was available for pathological examination, however, the process, though varying in extent from part to part, involved the gland as a rule in its entirety, and the apparent immunity of the upper and inner quadrant could not be substantiated.

Cysts were definitely detected on physical examination in fourteen cases. In ten cases there was doubt as to whether a cyst were present or not, and in three cases no cysts were found on gross examination of the specimen. In every case cysts were found on microscopic examination of the specimen. Two cases only showed diminution in the size of the mass from time to time. Two cases also gave the history of the discharge of a clear or watery fluid from the nipple. These symptoms, while extremely suggestive of the presence of a cyst, as pointed out by many writers, can be considered as of only confirmatory evidence. The absence of these symptoms can have little value in diagnosis on account of the relative infrequency of their occurrence.

Pain and tenderness on palpation are symptoms of considerable importance, in connection with the generally painless character of cancer of the breast. Twelve cases complained of pain and tenderness in the breast, and seven cases gave no evidence of this condition. In eight cases the presence or absence of this symptom was not recorded.

The nipple was retracted in three cases, normal in ten, and its condition was not recorded in fourteen cases, but may be assumed to have been normal. The retraction of the nipple is not a feature of this disease, and when present is probably of antecedent origin and of negative importance.

Adherence of the gland to the overlying skin or to the underlying fascia was not found in any case.

Axillary glands of palpable size were recorded as present in seven cases. They were noted as doubtful in two and absent in thirteen cases. The examination was not recorded in five cases. When present, palpable glands were frequently found in both axillæ, whether one or both breasts were involved in the disease.

The clinical picture presented by chronic cystic mastitis may be summarized as follows: An indurated mass of irregular contour and ill-defined margins is found, either diffusely pervading the mammary gland or localized most often in the outer hemisphere. Cysts are frequently observed and may be of considerable size. The mass is generally of slow growth, unless large cysts are present. It is generally, though not invariably, painful and tender to pressure, and often involves both breasts. Discharge from the nipple and diminution in the size of the mass occur but rarely. The disease is found either in single or married women and generally at a period shortly before the time of the menopause. The skin is not adherent, and the axillary glands are but slightly if at all enlarged.

Chronic cystic mastitis is to be distinguished from the following diseases in making a differential diagnosis: cancer, sarcoma, adenoma (villous papilloma or duct cancer of the English writers), the solid and cystic fibromata (adenofibroma), galactocele and retention cysts of traumatic origin, chronic and subacute mastitis after lactation, tuberculosis, syphilis, actinomycosis, retro-mammary disease, and foreign bodies in the breast. It is also to be distinguished from normal involution of the breast. Few of these conditions need special consideration, but attention may here be directed to the varied nomenclature employed by different writers in the description of tumors and diseases of the breast. The terminology of Gross[46] has been adopted in this paper, as being the one taught in the Surgical Department of the Harvard Medical School. By this classification the term "Fibroma" is used to denote the varied class of tumors in young adult breasts which take origin in the "periductal" tissue, and which are classed as adeno-fibromata or intracanalicular papillary fibroma by other writers. Adenoma in the Gross classification is used to indicate the tumors which present an obvious new formation of gland elements more or less typical in form, and of local character. These tumors are known as duct cancer or villous papilloma by English writers, and as proliferative or papillary cyst-adenomata in the German literature. They are of rare occurrence. Tumors of this class are with difficulty divided from certain advanced cases of chronic cystic mastitis. Papillary outgrowths of macroscopic size, such as occur in villous papilloma in the dilated ducts beneath the nipple, have been excluded from this series of cases. Such tumors almost invariably present the characteristic symptom of a bloody discharge from the nipple, and by their localized situation and limited extent can be distinguished clearly from the diffuse process in chronic cystic mastitis. Cases which present proliferative or papillary growths in the wall of a cyst can be distinguished only by the extent to which the process is judged to involve the gland. In other words, no sharp line can be drawn, either clinically or pathologically, between some cases of cyst-adenoma and chronic cystic mastitis.

The subacute and chronic suppurative processes which follow lactation are to be clearly distinguished from chronic cystic mastitis. They are accompanied by minute abscesses along the ducts, by more or less intense inflammatory reaction in the surrounding tissues, and by their tendency to progress to suppuration. Whether or no mild degrees of this affection predispose to later cystic mastitis, as held by Delbet and others, will be discussed below.

In nine cases amputation of the breast was performed by the surgeon in charge of the case. In fifteen a portion of the gland was resected, and in two the specimen was obtained from the autopsy table. The material thus obtained was examined with reference to the following points:

The fibrous tissue was invariably increased, and generally throughout the entire gland. It was rarely homogeneously distributed, but was more dense in certain areas or about certain lobules. In twenty-six cases the process was recorded as diffuse, in only one as discrete or local. The fibrous tissue varied from white to gray in color, and in many cases was of cicatricial consistency.

Cysts were present in every case. In two cases they were of microscopic dimensions only. In twenty-five they were readily seen on gross examination. The contents of the cysts were, as a rule, fluid and varied from clear watery fluid without color, through all shades of yellow and green to dark brown and almost black. Papillary growths appreciable on gross examination were not present in any case.

Lobular proliferation without cyst formation was noted in one or two cases on gross examination, as presenting an appearance of fine granules of softer consistency scattered through the dense fibrous tissue, particularly at the outer portions of the mammary gland.

Sections made and stained by the methods already detailed were examined with the microscope, and gave the following results:

The increased fibrous tissue of chronic cystic mastitis arises chiefly, if not altogether, from the interlobular connective tissue of the gland. Every case showed increase of the interlobular tissue, while in only eight could an increase of the periductal tissue be determined, and that of only insignificant extent. The interlobular tissue was as a rule distinctly poor in cells and the fibers were broad and deeply stained as in cicatricial tissue of an advanced age. The periductal tissue, while more cellular than the interlobular tissue, was not more cellular than in the normal breast, and in five cases marked evidence of hyaline degeneration of this tissue was apparent. In ten others slight degrees of hyaline change of the periductal tissue, especially in the portions nearest to the basement membrane of the acini, could be determined. In eleven instances hyaline changes in the interlobular tissue were observed. The distribution of the interlobular tissue was general throughout the gland, but especially surrounding the acini and ducts. Occasionally a collapsed duct was seen surrounded and apparently compressed by fibrous tissue.

Round cell infiltration is commonly regarded as evidence of an inflammatory process of a chronic nature. The same phenomenon appears, however, about a carcinoma of rapid growth, and is there attributed to the inflammatory reaction set up in the tissues as a result of the invasion of the malignant growth. Round-cell infiltration of the tissues was observed in twenty of the twenty-seven cases in this series. The situation of the infiltration was subject to a considerable degree of variation. In thirteen cases the round cells were most marked about the ducts and acini (Plate XX., Fig. 4), in five cases about the cysts (Plate XX., Fig. 3), and in two about the blood vessels. Cells of several varieties were present in this infiltration. Lymphoid cells were most frequently observed, and were present in every one of the twenty cases. Plasma cells were found in nineteen cases, and leucocytes in eight. Eosinophiles and mast cells were rarely seen. Two cases showed evidence, both clinically and pathologically, of suppuration occurring in a breast already the seat of chronic cystic mastitis. In these instances the round-cell infiltration and the presence of leucocytes were by far more marked than in the others. In several instances round-cell infiltration was most conspicuous in close proximity to the areas of most vigorous epithelial proliferation. In other cases this relation was not demonstrable. From the examination of this series of specimens the writers were forced to conclude that the round-cell infiltration plays a secondary and subordinate part in the lesions of chronic cystic mastitis. It appears to be chiefly due to the local irritation which is caused by either degenerative or productive processes in the epithelium of the acini and cysts. This view is at variance with the greater number of the published opinions upon the subject, but in no other way can the variable and inconsequent appearance of round cells be explained. It is certain that no evidence could be found in any of this series of cases to justify the assumption of Binaud,[6] Nocard,[28] and others that a bacterial infection of the ducts is responsible for the etiology of this disease.

Endothelial cells, presumably derived from the lymph spaces, were conspicuous in three of these cases. These cells were of large size and occupied a position in the connective tissue near to the wall of cysts of considerable size. This appearance has been noted by Rogowitsch[36] and by Maly,[24] and attributed to edema and imbibition of the fluid by the cells. In our cases this explanation for the phenomenon seemed a satisfactory one, in view of the presumable blocking of lymphatics by the enlargement of the cyst.

The amount of fat in these specimens varied considerably, and seemed to depend largely upon the habit of the individual. The fat lying in the meshes of the interlobular connective tissue appeared to be diminished in sixteen cases, while in eleven no such diminution could be demonstrated. (Plate XX. Fig. 2.) The delicate fibrous tissue which enmeshed the fat cells was more cellular in character than the solid masses of fibrous tissue. It seemed possible that the increase of the interlobular fibrous tissue which occurs in this disease was, in a measure at least, at the expense of the interlobular fat and coincident with its disappearance. Interlobular fat was totally lacking in only one case of this series. Its presence, therefore, as pointed out by Rolleston,[40] would serve to distinguish this condition from the encapsulated fibromata in which no fatty tissue is found.

Blood vessels were rarely present in large numbers, and were generally small in size. Evidence of endarteritis was very rarely seen. Few of these cases, however, were of an age to show marked senile arterial changes, and women are understood to be less liable to this disease than men.

The large ducts of the breast gland do not present marked variation in appearance in specimens of chronic cystic mastitis. Occasionally they are obviously infolded and compressed by the dense interlobular connective tissue. Often they do not show any evidence of this phenomenon. The periductal tissue of the larger ducts is normally small in amount at this stage of the breast development. The epithelium is generally two or more layers in thickness, but is ill-defined, and apparently in an atrophic condition. No evidence of proliferation, such as occurs in the adenomata which arise from this tissue, could be detected. Round-cell infiltration was occasionally present about the larger ducts, but was quite inconstant in its appearance and amount. Accumulation of degenerated epithelium and granular débris in the lumen of the larger ducts was occasionally noted.

The number of acini in each lobule was notably increased in thirteen cases and slightly increased in five. In several instances this increase was sufficient to suggest the proliferation of acini in the pregnant or lactating breast. (Plate XX., Fig. 2.) In many instances the number of acini was decidedly diminished. Variation in this respect occurred in the same breast, and even in the same section, some acini being increased and some diminished.

The peri-acinal connective tissue was never markedly increased. Occasionally it was diminished, and rarely it presented a degree of hyaline degeneration. The peri-acinal tissues, however, were frequently infiltrated with round cells, and this change, except in the two cases of suppuration, was more marked in the regions where the most increase of acini occurred, and particularly where proliferative changes in the epithelium of the acini could be demonstrated. The epithelium of the acini exhibited a great degree of variation. Where the interlobular connective tissue was greatly increased and the acini diminished in number atrophic forms of epithelium were seen. The cells were small, irregular, flattened, and the nuclei small and with poorly staining chromatin. These regions were regarded as the unaffected portions of the gland which were undergoing normal involution.

When increase of the acini in a lobule occurred, and rarely when the number of acini in each lobule was diminished, the epithelium presented a more varied picture. One or two layers of cells were commonly found, and occasionally a more vigorous proliferation of the epithelium could be demonstrated. The cells in the proliferated areas stained well, showing a fine to coarse granular protoplasm, rarely vacuolated. The nuclei showed abundant chromatin, staining sharply as a rule, but varying somewhat from cell to cell. Mitoses were rarely observed. (Plate XXII., Fig. 1.) Between these two degrees of epithelial growth an infinite number of intermediate stages could be observed.

In the majority of instances a tendency to the formation of cystic dilatation was to be observed. No specimen of this series gave evidence of direct invasion of the connective tissue by proliferation of the epithelium of undilated acini.

Cysts obviously derived from the larger ducts could not be demonstrated in these cases. The epithelial lining of the cysts did not present the characteristics of the ductal epithelium as it is observed in adenoma derived from these tissues. The large cysts, however, in which the epithelial lining membrane had degenerated and disappeared, did not admit of any deduction with regard to their point of origin.

In the main, the cysts appeared to be derived from the smaller ducts and acini. In several cases a definite picture of the dilatation of the duct and acini of a single lobule (Plate XXI., Fig. 1) could be seen. In such cases the epithelial covering of the dilated duct was of a similar character to that of the cystic acini. For this reason a division between these two forms of cysts is impracticable, if not impossible. The dilatation of all the acini of a single lobule was observed in fifteen different specimens, and points strongly to compression of a duct as the cause of the cystic dilatation. (Plate XXI., Fig. 2.)

The epithelial lining of the cysts was subject to the greatest variation, and that without regard to the size of the individual cyst. Atrophic forms of epithelium, low cuboidal or flattened cells, with poorly staining nuclei and vacuolated protoplasm, occurred in cysts of all sizes. Epithelial cells of a normal acinal type, one or two layers deep, with well stained nuclei and finely granular protoplasm, were also found in cysts of varied sizes. Hypertrophic or proliferative epithelium of the high columnar form so frequently described in cystic disease of the breast were found in nearly every case. These cells have a coarse or fine granular protoplasm and sharply staining nuclei. They often show evidence of secretion at the extremity of the cell nearest to the lumen. They occur in cysts of all sizes, and were found in early acinal dilatation. (Plate XXII., Fig. 1.) For this reason they are regarded by the writers as derived from the acinal epithelium, rather than from the ducts. Paul[27] regards these cells as of slow growth and innocent nature. Sasse,[38] Schimmelbusch,[39] and others consider them evidence of tumor formation of the polycystoma or cyst-adenoma variety. The writers prefer to regard them as a stage of proliferation of the epithelium, the significance of which will later be discussed.

Papillary outgrowths of epithelium of this columnar form were present in thirteen cases. Papillary epithelial structures with a connective tissue stalk were produced in several instances by the confluence of two or more adjacent cysts, on the rupture of the delicate connective tissue wall between them. (Plate XX., Fig. 6.) Similar structures without a connective tissue framework were apparently produced in individual cases by the simple proliferation and heaping up of the epithelial cells. (Plate XXII., Figs. 2 and 3.)

A higher degree of epithelial proliferation in cysts was found when papillary outgrowths from the walls met and united in the center or anastomosed laterally, forming masses of epithelium without connective support. (Plate XXII., Fig. 4.) In these cell masses the tendency to the formation of a gland lumen and the arrangement of the epithelial cells radially around this space could be demonstrated beyond all doubt. This form of growth the writers, for lack of a better term, have designated as "adenomatous proliferation." This phenomenon has been described by Paul[27] as the growth of carcinoma infiltrating a preëxisting cyst. That it approaches carcinoma is not to be denied, but in twenty-seven cases it was present seven times, and in not one of these cases could evidence of carcinoma be elsewhere obtained.

That these degrees of proliferation are progressive and lead one to another is proved by their occurrence in this series of twenty-seven cases. Twenty-four showed columnar epithelium in cysts; thirteen of these twenty-four showed papillary epithelial growth. In seven of the thirteen cases of papillary growth, "adenomatous" forms of proliferation were observed. No adenomatous proliferation occurred in cases that did not elsewhere show papillæ, and no papillæ occurred except in cases which showed columnar cells. For these reasons the writers disagree with Paul's conclusions that the hypertrophic columnar cell is an innocent and "resting" stage of the epithelium.

Evidence of active cell growth, as proved by mitoses, was present in only seven of the entire twenty-seven cases, and was found both in the cystic and acinæ types of proliferation. It must be borne in mind, however, that mitosis is a phenomenon of little negative value on account of the rapidity of the process, and that active and rapidly growing carcinomata often show only one or two mitoses, and that after the examination of many sections.

The contents of the cysts in these cases was subject to much variation. Fluid contents varied in consistency from watery to mucilaginous, and in color from colorless to green, brown, and black. Epithelial cells were frequently observed free in the cavity of the cyst. In some cases they were obviously degenerated; in other cases, well preserved and nearly filling the lumen of the cyst, fixed as it were in a coagulum of homogeneous material. Many cells showed granules of pigment, similar in every way to blood pigment; but no active hemorrhage into a cyst could be made out. Leucocytes and an occasional red corpuscle were seen; but by far the greater number of cysts were filled with a fine granular débris which appeared to be due to the degeneration of the epithelial cells cast off from the wall of the cyst. Mention has already been made of papillary growths in cysts and adenomatous proliferation of the epithelium.

To sum up the histological changes which are found in cases of chronic cystic mastitis, the following features of the process are to be recognized:

The increase of fibrous tissue is derived from the interlobular connective tissue of the gland. It is coarsely fasciculated and of cicatricial consistency. Round cell infiltration is frequently observed, but appears to be only of secondary importance in the pathology of the disease.

The large ducts show little change in their appearance. They occasionally appear to be compressed by fibrous tissue.

The number of acini in each lobule varies considerably, and their lining epithelium is in various stages of proliferation, although rarely showing any atypical growth.

Cysts are chiefly produced by dilatation of the smaller ducts and acini. The epithelial lining is subject to the greatest variation, from epithelial cells of the normal acinal type to hypertrophic epithelium of the high columnar form. More advanced degrees of proliferation are seen in papillary outgrowths of the epithelium, with or without a connective tissue stalk. A still further stage of proliferation is seen in the formation of masses of epithelial cells in cysts by the fusion of papillary growths, and the production of cell areas of an adenomatous character.

The progressive nature of the epithelial changes in chronic cystic mastitis is a feature of the greatest importance. The nature of the cyst formation, and the part played by the epithelium in its production in this disease, has been the subject of much discussion.

Reclus[33] believed that the cyst was of epithelial origin. Koenig[20] and the modern French school[6, 15] maintain the inflammatory etiology of the disease. Schimmelbusch[39] pronounces it a tumor formation (Polycystoma). Sasse[38] gives recognition to two classes — (*a*) Involution and inflammatory cysts; (*b*) Polycystoma.

The difficulty in drawing such a distinction as is made by Sasse is at once apparent. Every degree of variation, from simple involution to extreme proliferative cyst growth, occurs in our twenty-seven cases.

The decision of this question demands the reconsideration of several characteristics of the process, both clinical and pathological. The inflammatory and bacterial origin invoked by the modern French writers (Binaud,[6] Delbet,[15] etc.) is not supported by the histological characteristics of the tissue. Whether or not such a condition precedes the appearance of the disease cannot be authoritatively stated. No evidence exists, however, in the clinical histories to support this view.

Inflammatory changes other than bacterial in origin cannot readily be excluded in the etiology of this condition.

The main characteristic of the process by which a gland or member which is no longer of functional value to its possessor undergoes atrophy is by the disappearance of the active differentiated elements, and their replacement by fibrous tissue. This process is seen in the uterus and in the breast which undergoes normal involution.

In the breast, however, a lack of coördination between this atrophy and the fibrous tissue increase is not infrequent. Obstruction of the larger ducts by compression or by the collection in them of degenerating epithelium, and the resulting formation of retention cysts, may be considered the explanation of "involution cysts" which receives most general acceptance by German, English, and American writers.

The transition from this stage of the disease is a gradual one to the condition in which extensive proliferation of the cyst epithelium occurs, and papillary and adenomatous masses are found in the cyst cavities. The writers have been obliged to use the term "adenomatous proliferation" to describe the appearance of the epithelium in certain of these cases. The occurrence of intermediate stages, however, and particularly the widespread distribution of the adenomatous areas in different lobules of the breast, or even in both breasts, seem to them reason sufficient to reject the term of polycystoma, or cyst-adenoma (Sasse, Schimmelbusch, Maly[24]) on account of the suggestion of a local tumor growth, which the name implies.

The epithelial proliferation in these cases is considered by the writers to be the reactionary hyperplasia of epithelium placed under abnormal conditions. It matters little whether it is classed as "adenomatous proliferation," or dignified by the name of "polycystoma." The progressive nature of the epithelial growth is the feature which must be recognized. Whether the well-known instability of the breast epithelium renders it more liable to react in this exaggerated way to causes which in other organs would not thus affect the epithelium can be only a matter of speculation. The fact is certain, however, that progressive changes in the epithelium of the breast gland are found in chronic cystic mastitis, which closely approach the border line between inflammatory hyperplasia and new growth. That these progressive changes of the epithelium occasionally go beyond this border line, and present the characteristics of actual new growth and that of a malignant nature, can be demonstrated.

Carcinoma was present, in addition to the lesions of chronic cystic mastitis, in three cases of this series. The diagnosis of carcinoma in these three cases was based upon the following histological features, which were not found in the twenty-seven cases of simple chronic cystic mastitis: (1) Extreme degrees of proliferation of the epithelium were present. (2) Irregularity in the size and shape of the epithelial cells was conspicuous. (3) Irregularity in the size and shape of the nuclei of the epithelial cells was present. (4) Mitosis was frequently observed, and the mitotic figures were occasionally atypical in form. (5) Evidence was present of the infiltration of the epithelial cells into the connective tissue stroma, in masses of irregular shape and without a limiting membrane.

The clinical features of these three cases differed in only a slight degree from those of the uncomplicated cases. One patient was forty-three years old, one thirty-nine, and one of unknown age. Two cases were married, one was single. One of the married women had borne and nursed three children, and one was childless. No history of trauma or of breast abscess was obtainable in any case. One patient had passed the menopause, the other two gave no evidence of uterine disease or menstrual disorder. The disease had existed only from one to three months in these three cases. The progress of the disease was gradual in every case. In two cases only one breast was involved. In the other both breasts were affected at different periods.

On clinical examination the indurated area occupied the outer portion of the breast in every case, and varied in size up to a mass the size of a hen's egg. Cysts were recognized on physical examination in one case by the nodular and elastic feeling of the tumor. No diminution of size was noted in any case, but a bloody discharge from the nipple was recorded in one case. Tenderness was not observed of any marked degree in any case. The nipple was normal in appearance in all three cases. Adherence of the skin to the indurated area was noted in two cases. Palpable axillary glands were not found in any case. Two patients had the complete operation done for cancer of the breast, with amputation of the breast and dissection of the axilla. The axillary glands in one of these cases failed to show metastasis. In the other case the condition of the glands could not be determined. These two cases of amputation have given no sign of recurrence up to the present time (April, 1903), ten months in each case after the operation. The other case had the tumor resected, without removal of the entire gland, and recurrence took place three years later, in the region of the scar.

By this brief analysis of the clinical features of the three malignant cases, it is evident that no special signs or symptoms serve to distinguish with certainty between cases of simple chronic cystic mastitis and those which have progressed as far as carcinoma. Adherence of the skin to the induration and a bloody discharge from the nipple may surely be regarded as suspicious symptoms, but it would be dangerous to rely upon these two symptoms on account of the inconstancy of their appearance.

The gross examination of the specimens removed at operation in these three malignant cases failed to show positive evidence of the presence of carcinoma. They all presented the characteristic increase of fibrous tissue and multiple cyst formation of chronic cystic mastitis, and only on microscopic examination could their true nature be determined.

All three cases presented the histological appearances of chronic cystic mastitis as above described. The degree of epithelial proliferation in the cysts was, however, the most conspicuous feature of the specimens. Papillary and adenomatous types of proliferation were present in every case, similar to the degree of epithelial proliferation described in the simple cases in seven instances. In addition to these lesions, however, and as a rule in close proximity to them, were found the characteristic lesions of carcinoma above described. Numerous areas were present in each specimen in which the gradations between adenomatous proliferation and carcinoma were so delicate as to be practically indistinguishable. Only the extremes of the two conditions could with certainty be identified.

The areas of undoubted carcinoma in these specimens were all of one and the same general type. Epithelial cells of all sizes and degrees of irregularity were heaped together in masses without connective tissue support. Here and there, in the midst of the epithelial cells, spaces were found, around which the cells were placed in radial arrangement, with their long axis perpendicular to the margin of the space. Vacuoles and secretion bodies were frequently observed between the nucleus and the free border of the cell. These spaces are commonly described as the attempt of the gland cells to arrange themselves about a lumen, and carcinoma which presents this picture is commonly known as adeno-carcinoma. (Plate XXII., Fig. 5 and 6.)

Serial sections of these tissues showed in several instances that cysts with adenomatous proliferation, forming solid columns of epithelial cells, were directly continuous with acini and ducts, the lining epithelium of which was of the high columnar type. This observation we considered definite proof that progressive changes from columnar to papillary and adenomatous proliferation of the breast epithelium occur in chronic cystic mastitis.

In other cases cell masses of similar appearance were found without relation to any ducts or acini, and were then regarded as areas of infiltration of the lymphatics by masses of carcinoma.

Round-cell infiltration was present in the three malignant cases, as well as in the simple ones. In the malignant cases the plasma and lymphoid cells, which constituted the greater part of the infiltration, were found in close proximity to the areas of most marked epithelial proliferation, and especially about the areas of undoubted carcinoma.

The relation of chronic cystic mastitis to cancer has long been a matter of discussion. Beadles[3, 4] found evidence of chronic mastitis in the breast tissue of one hundred cases of carcinoma of the breast in the outlying portions of the gland which were not involved in the tumor growth. It is not clear, however, that the lesions which he described as chronic mastitis were more than the normal involution of the declining breast. It would be futile to deny the possible coexistence of the two lesions, inasmuch as at the time of the menopause the breast is notoriously liable to both forms of disease. That chronic cystic mastitis precedes and predisposes to cancer is not thereby established. So far as schirrhus cancer is concerned, no case was found in which the lesions of chronic cystic mastitis were present during the three years which have elapsed since the first specimens for this investigation were collected. The writers can only call attention to the lack of evidence in this series of cases to justify the association of the two diseases. The relation of chronic cystic mastitis to adeno-carcinoma, on the other hand, is most conspicuous. All stages of progression between simple hyperplasia of the gland epithelium and actual adeno-carcinoma can be found. Step by step it is possible to follow the proliferation of the epithelium through the columnar and papillary forms to adenomatous proliferation and to adeno-carcinoma.

Adeno-carcinoma is generally admitted to be one of the less malignant types of cancer, and by its large bulk little danger exists of its escaping the notice of the patient until too late for operative relief. It is important, however, that its true cancerous nature be appreciated, and that the diagnosis of chronic cystic mastitis should not be allowed to quiet the apprehension of the patient, and especially of her physician.

The treatment of chronic cystic mastitis is therefore clear. Operation is indicated in almost every case, not only on account of the possible malignant tendency of the disease itself, but also on account of the entirely independent disease which its presence may mask. In certain very early cases and those with slight involvement, in which the presence of a cyst makes the diagnosis positive, delay in the application of the operation may be permissible. Increase of the indurated area, however, should be considered a positive indication for operation. The operation to be advised is the subcutaneous excision of the whole of the breast gland, leaving the nipple undisturbed. The Thomas incision below the breast makes this procedure possible with the minimum of deformity. When adenomatous proliferation is found on microscopic examination of the specimen, the case is to be regarded as one of extreme gravity, and frequent examinations by the surgeon are demanded, to ensure the early detection of a possible recurrence.

CONCLUSIONS.

1. The name of "Chronic Cystic Mastitis" (Koenig) is the most satisfactory of the many terms used to describe the condition of fibrous tissue increase and cyst formation in the female breast at the time of the menopause.

2. The use of the term "mastitis" should not imply an inflammatory process due to bacterial infection, but rather an increase of fibrous tissue of other than local tumor origin.

3. Increase of the fibrous tissue of the breast is the normal condition in the stage of decline of the gland at the time of the menopause.

4. The formation of cysts and the proliferation of the epithelium of the cysts and acini are the striking features of the disease.

5. Variations of the epithelium are found in this disease, from atrophic forms, through columnar cells, to papillary proliferation and cell masses of an adenomatous type.

6. The transition of adenomatous types of proliferation to carcinoma of the adeno-carcinoma type occurs in a limited number of cases.

7. Transition to other types of cancer was not observed.

8. The danger of the transition of chronic cystic mastitis to adeno-carcinoma is sufficient to make the removal of the entire gland advisable in all but very early and slight degrees of the affection.

9. The operation to be advised is as a rule the subcutaneous resection of the entire gland without removal of the nipple. This is best effected by means of the Thomas incision under the fold of the breast.

SUMMARY OF CASES.

Case I. — M.G.H., Dr. Codman, 47, married, one child, three weeks, both breasts—left breast lower outer quadrant, right breast lower inner quadrant, cystic, tender. Operation, resection of cysts and indurated areas in both breasts. No recurrence one and one-half years.

Pathology: Cysts, columnar proliferation, increase of acini in few lobules, lobular cyst formation.

Case II. — M.G.H., Dr. Warren, 45, married, four children, two years, induration size hen's egg in upper outer quadrant of one breast, slightly tender, one small axillary gland. Operation, resection. No recurrence one and one-quarter years.

Pathology: Cysts, columnar proliferation, proliferation of acinal epithelium.

Case III. — M.G.H., Dr. Warren, 45, single, five months, induration outer lower quadrant left breast (right breast subsequently affected), cystic, not tender, nipple retracted, axillary glands enlarged. Operation resection and removal of axillary glands. No recurrence one and one-quarter years.

Pathology: Cysts, columnar, papillary, and adenomatous proliferation; increase of acini in lobules, acinal proliferation. Glands from axilla not involved. Lobular dilatation.

Case IV. — M.G.H., Dr. Porter, 41, married, three children, two miscarriages, two months, "tumor" in outer half of breast, tender, nipple inverted, yellow discharge from nipple. Amputation, no recurrence one and one-quarter years.

Pathology: Cysts, columnar proliferation, lobular dilatation, increase of acini in lobule, acinal proliferation.

CASE V. — M.G.H., Dr. Beach, 42, married, five months, mass size egg in upper outer quadrant left breast, not tender, cystic. Amputation, no recurrence one and one-quarter years.

Pathology: Cysts, columnar, papillary, and adenomatous proliferation, lobular dilatation.

CASE VI. — M.G.H., Dr. Warren, 37, married, no children, five months, nodular indurations in both breasts, noted diminution in size after onset, not tender, small gland in axilla. Operation, resection of indurated mass in right breast, and removal of axillary gland. Subsequent history unknown.

Pathology: Small cysts, no proliferation in cysts, slight increase acini in lobule, slight lobular proliferation.

CASE VII. — M.G.H., Dr. Conant, 49, married, two children, four months, "tumor" in left breast size silver dollar (right breast previously affected), not tender. Amputation, no recurrence in left breast, right breast recurrence of cyst formation one and three-quarters years.

Pathology: Cysts, lobular dilatation, columnar proliferation. Increase of acini in lobule. Lobular proliferation.

CASE VIII. — M.G.H., Dr. Conant, 43, married children (?), one week, nodular thickening both breasts, most in left, cystic, slightly tender, operation resection, both sides. No recurrence one and three-quarters years.

Pathology: Cysts, columnar proliferation.

CASE IX. — M.G.H., Dr. Conant, 35, single, duration unknown, mass lower half left breast, cystic (?), right breast also affected. Operation, resection of left breast. No recurrence one and one-half years in left. Induration in right breast increasing and obviously cystic.

Pathology: Cysts, columnar, papillary, and adenomatous proliferation, increase of acini in lobule, acinal proliferation.

CASE X. — Private case, Dr. Warren, 40, single, ten weeks, mass upper outer quadrant left breast, cystic, not tender, right breast similar trouble, resected four months before. Operation, resection of indurated mass. No recurrence three years.

Pathology: Cysts, columnar, papillary, and adenomatous proliferation, increase of acini in lobule, lobular dilatation, acinal proliferation.

CASE XI. — Private case, Dr. Cobb, 48, married, two children, two weeks, tender, indurated mass left breast, "tumor," right breast normal. Operation, amputation. No recurrence one year.

Pathology: Cysts, columnar proliferation, acinal proliferation.

Case XII. — Private case, Dr. Anna Richardson, 42, single, two weeks, mass upper outer quadrant right breast, nodular, not tender. Left breast, similar process less extensive. Operation, resection right breast. Subsequent history unknown.

Pathology: Cysts, lobular dilatation, columnar, papillary, and adenomatous proliferation, acinal proliferation.

Case XIII. — Autopsy case, death from cancer of uterus. 47, married, one child. No clinical history.

Pathology: Cysts, lobular dilatation, columnar, papillary, and adenomatous proliferation, slight increase of acini in lobule, acinal proliferation.

Case XIV. — M.G.H., Dr. Elliott, 32, single, five weeks, mass size egg upper outer quadrant left breast, cystic, tender. Operation, excision. Subsequent history unknown.

Pathology: Cysts, increase of acini in lobule, acinal proliferation.

Case XV. — M.G.H., autopsy case, 32, married, four children, lactation interrupted, death from pericarditis, no clinical history.

Pathology: Cysts, columnar and papillary proliferation, increase of acini in lobule, acinal proliferation.

Case XVI. — Private case, Dr. M. H. Richardson, 48, married, three children, duration (?), large inflammatory mass, cystic and suppurative. Operation, amputation. Subsequent history unknown.

Pathology: Cysts, low columnar proliferation, a case of extensive suppuration in a previously cystic breast.

Case XVII. — Private case, Dr. Warren, 48, married, no children, irregular catamenia, two days' duration, hard mass upper outer quadrant, cystic, tender, "tumor." Operation resection of indurated area. No recurrence one year.

Pathology: Cysts, columnar proliferation, lobular dilatation, acinal proliferation.

Case XVIII. — Private case, Dr. M. H. Richardson, 45, married, no children, two miscarriages, one year, mass size egg, right breast, cystic. Amputation. Subsequent history unknown.

Pathology: Cysts, lobular dilatation, columnar and papillary proliferation, increase of acini in lobule, acinal proliferation.

Case XIX. — Private case, Dr. Cabot, age unknown, married, children(?), four days, indurated cystic mass in one breast. Resection. Subsequent history unknown.

Pathology: Cysts, lobular dilatation, columnar and papillary proliferation, slight increase acini in lobule, acinal proliferation.

Case XX. — M. G. H., Dr. Elliott, 26, single, irregular catamenia, three years' duration, mass size olive, lower outer quadrant left breast, cystic. Operation, resection. No recurrence one year.

Pathology: Cysts, lobular dilatation, columnar and papillary proliferation, increase of acini in lobule, acinal proliferation.

Case XXI. — M. G. H., Dr. Richardson, 25, married, no children, one week, diffuse induration left breast, tender. Operation, resection of indurated mass. No recurrence one year.

Pathology: Cysts, slight lobular dilatation, columnar proliferation, increase of acini in lobule, acinal proliferation.

Case XXII. — Private case, Dr. Warren, 45, married, no children, duration unknown, cystic tumor, size English walnut, lower outer quadrant of one breast, tender. Operation, exploratory incision, resection of indurated area. No recurrence ten months.

Pathology: Cysts, lobular dilatation, columnar, papillary, and adenomatous proliferation, increase of acini in lobule, acinal proliferation.

Case XXIII. — Private case, Dr. Brewster, 30, married, one child, four years' duration, thrown from carriage injuring right breast, swelled up immediately, subsided, and has increased intermittently since then. Right breast diffusely enlarged, indurated, tender. Operation, amputation. Subsequent history unknown.

Pathology: Microcysts, increase of acini in lobule, acinal proliferation, lobular dilatation.

Case XXIV. — Private case, Dr. Porter, past middle life, married, children, one and one-half years, diffuse induration of breast, cysts, not tender, other breast previously removed for similar trouble. Operation, excision. Subsequent history unknown.

Pathology: Cysts, lobular dilatation, columnar and papillary proliferation, acinal proliferation.

Case XXV. — Private case, Dr. Scudder, 26, single, mastitis in infancy, bilateral, left nipple retracted since infancy, diffuse induration left breast, nodular, cystic, not tender. Operation, resection. No recurrence three and one-half months. Right breast, similar affection, resected three and one-half months later.

Pathology: Cysts, lobular dilatation, columnar and papillary proliferation, slight increase of acini in lobule, acinal proliferation.

Case XXVI. — M. G. H., Dr. Warren, 52, single, menopause one year ago, four weeks, indurated mass in central portion left breast, tender, "tumor," right breast normal. Operation, resection. Subsequent history unknown.

Pathology: Cysts, columnar proliferation, suppuration in cysts.

Case XXVII. — Private case, Dr. Brewster, 40, married, two children, eight months, diffuse induration of right breast, clear, watery discharge from nipple. Operation, amputation. Subsequent history unknown.

Pathology: Microcysts, columnar proliferation, increase of acini in lobule, acinal proliferation.

Case XXVIII. — M. G. H., Dr. Harrington, 43, married, three children, two months, nodule size walnut, outer half left breast, slightly tender. Operation, excision. Recurrence three years. Right breast also affected.

Pathology: Cysts, columnar, papillary, and adenomatous proliferation, and adeno-carcinoma.

CASE XXIX. — Private case, Dr. Vickery, age unknown, single, one month, indurated mass lower outer quadrant right breast, cystic, not tender, adherent to skin. Operation, amputation. No recurrence ten months.

Pathology: Cysts, columnar, papillary and adenomatous proliferation and adeno-carcinoma.

CASE XXX. — M. G. H., Dr. Mixter, 39, married, no children, three months' duration, diffuse induration and nodule in upper outer quadrant left breast, tender, bloody discharge from nipple, skin adherent. Operation, excision. No recurrence ten months.

Pathology: Cysts, columnar, papillary, and adenomatous proliferation and adeno-carcinoma.

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DESCRIPTION OF PLATES.

PLATE XX.

Fig. 1. Normal involution of the breast in a woman of fifty-eight years of age, from an autopsy case. Low power.

Fig. 2. Increase of acini in a lobule. Persistence of interlobular fat. Case XIX. Low power.

Fig. 3. Round-cell infiltration in the wall of a cyst. Case IV. Low power.

Fig. 4. Round-cell infiltration around a small duct, in the lumen of which exfoliated epithelium has collected, and a slight degree of proliferation has occurred. Case XXV. Low power.

Fig. 5. Involution cyst with granular contents. Case XI. Low power.

Fig. 6. Cysts derived from acini, with high columnar epithelium.

Confluence of adjacent cysts by rupture of dividing wall. Case X. Low power.

Plate XXI.

Fig. 1. Cystic dilatation of the acini and duct of a single lobule. Case VII. Low power.

Fig. 2. Cystic dilatation of a single lobule with round cell infiltration, Case IV. Low power.

Plate XXII.

Fig. 1. Proliferation of acinal epithelium in columnar form. Case XVIII. X 250.

Fig. 2. Proliferation of columnar epithelium of a cyst in papillary form without supporting stalk of connective tissue. Case XXIV. X 250.

Fig. 3. Multiple acinal cysts with high columnar epithelium and extensive papillary proliferation. Case XXIV. X 125.

Fig. 4. Adenomatous proliferation of epithelium of a cyst. Fusion of papillary outgrowths. Case III. X 250.

Fig. 5. Adeno-carcinoma. Masses of proliferated epithelium in the walls of cysts and forming solid bodies of cells in which gland lumina are present. Case XXVIII. Low power.

Fig. 6. Adeno-carcinoma. Masses of epithelial cells in which gland lumina are present with cells arranged radially around them. Case XXIX. X 250.

The photo-micrographs for this article were made by Mr. L. S. Brown at the Massachusetts General Hospital.

BACTERIOLOGY OF THE FIBRINOUS EXUDATE IN SEPTIC PERITONITIS.

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The presence of fibrin in acute infections of the peritoneal cavity is so frequent that it has seemed desirable to make a series of observations, in order to determine whether bacteria are present or absent in the fibrin. There is a difference of opinion among surgeons in regard to the treatment of the fibrinous exudate in operations. One group maintains that the fibrin may be left without harm, while another says it should always be removed. Finney says "the intestine should be thoroughly wiped off, in order to remove the flakes of partially organized lymph. It should be done thoroughly and conscientiously, however, as upon this, in a great measure, depends the success of the operation." (Finney, Johns Hopkins Bull., July, 1897.)

By presenting the following series of cases, I have endeavored to show that the organism causing the infection is, in most cases, present in the meshes of the fibrinous exudate.

METHOD OF EXAMINATION. — Cover glasses were taken from the fluid exudate and, in some cases, from the field of cleavage between the fibrin and the surface of the intestine. Cover glasses were then stained by Gram's method, methylene-blue, and eosin. (Smith, in Mallory and Wright's Path. Tech., p. 140.) Cultures on blood serum were taken from the same sources as the cover glasses, and from these dilutions were made. In some cases the presence of organisms of the colon group required the cultures on sugar agar. Plate cultures were also used when the variety of organisms was great. Anaerobic cultures were also made according to Wright's method. (Mallory and Wright, Path. Tech.)

METHOD OF OBTAINING THE FIBRIN. — By means of a director and a smooth-toothed forceps, the fibrin was peeled off the surface of the intestine and placed at once in ninety-five per cent alcohol. The fibrin was imbedded in paraffin by the usual method. The preparations hardened in Zenker's fluid contained so much precipitate that the sections were confusing.

METHOD OF STAINING. — Several methods were used. In some instances one stain worked to advantage, while in others it proved a failure. The following stains were found to be the most useful:

1. Carbol-thionin. (Nicolli.) (Hewlitt, Man. of Bacteriol., p. 76.)
2. Gram, eosin, methylene-blue. (Smith, Mallory and Wright, Path. Tech., p. 140.)
3. Gram and Aurantia, two per cent. (Smith.) This stain was suggested to me by Dr. W. H. Smith.
4. Carbol-fuchsin and Gram. (Flexner, Johns Hopkins Bull.)
5. Carbol-thionin and Gram. (Hewlitt, Man. of Bacteriol., p. 80.)

Nineteen cases of acute septic peritonitis were available for the purposes of this investigation. The clinical and pathological features of these cases were as follows:

CASE I. — *History:* Cancer of rectum, seven months' duration. Operation, inguinal colotomy. Second operation, nine days later. Resection of coccyx, with excision of rectum and lower end of sigmoid flexure.

Autopsy: General fibrino-purulent peritonitis. A portion of the remaining part of the rectum presented a dirty-gray appearance. It was destitute of mucous membrane and of a large portion of the muscularis. No perforation was found.

Smear preparation: Innumerable streptococci, leucocytes, and shreds of fibrin were found. Non-Gram-staining small bacilli.

Cultures on blood serum and sugar agar showed streptococci and a bacillus of the colon group.

Microscopic examination of fibrin and of peritoneum shows complete necrosis of the tissues, with no cell elements remaining. Innumerable streptococci and a great variety of bacilli. Sections were stained by Gram's method, eosin and methylene blue, also by Gram's method and Aurantia Plate XXIII., Figs. IV. and V.

CASE II. — Man, aged 42. *History:* Acute abdominal symptoms thirty-six hours before operation. Leucocytosis, 14,000. Diagnosis, appendicitis. Operation, abdominal cavity contained a small quantity of yellowish-brown fluid. Culture from this fluid was sterile. Appendix: Free surface injected and rough. Mesentery thickened. Second incision was made at the outer border of the left rectus, and the bowel accidentally punctured. The intestinal wound was closed with Lembert sutures, and the abdominal cavity closed without drainage. Death on the third day after operation. Symptoms of peritonitis appeared twelve hours after operation.

Autopsy: Acute fibrino-purulent peritonitis. Pelvic cavity contained a slight amount of reddish fluid. Serosa of the liver markedly injected. An adherent, fibrinous, reddish material is present along the coils of the intestine, and is very adherent in the lower abdomen. In the pelvis, distinct patches of different fibrinous material are present.

Smear preparation: Leucocytes. Vast number of bacteria, among which are a few micrococci.

Cultures on blood serum and sugar agar show bacilli of the colon group.

Fibrin: Granular fibrillæ. Leucocytes and several medium-sized bacilli. These sections were hardened in formalin and stained with carbol-thionin.

CASE III. — Woman, aged 31. Operation for increasing symptoms of intestinal obstruction of five weeks' duration. No evidence of peritonitis found at operation. Second operation the following day. Mixter tube placed in the jejunum. Death on the third day.

Autopsy: General peritonitis. No free fluid. Intestines injected. Dark and dull on the surface, with adherent fibrinous shreds.

Smear preparation: Many leucocytes, some lymphocytes, and endothelial cells. Many large Gram-staining capsulated bacilli. Morphologically this organism is considered to be the Bacillus aerogenes capsulatus of Welch. Also a large number of small, non-Gram-staining bacilli and a few short chains of streptococci.

Cultures from the peritoneal cavity on blood serum and sugar agar showed a small bacillus of the colon group. No anaerobic culture made.

Fibrin: Fibrillæ, leucocytes, and plasma cells. Several large capsulated Gram-staining bacilli.

CASE IV. — Woman, aged 56. *History:* Acute abdominal symptoms of eight days' duration. Patient was moribund on reaching the hospital, and no operation was performed. Leucocytosis, 38,000.

Autopsy: Gangrenous appendicitis. Diffuse fibrino-purulent peritonitis. The distal portion of the appendix is gangrenous, and has sloughed off. The abdominal cavity contained about 200 cc. of dirty brown opaque fluid. Fibrinous adhesions between the coils of intestine. In other places the intestine was covered with a fibrinous exudate.

Smear preparation: Leucocytes. Many large capsulated bacilli, Gram-staining. Several small, non-Gram-staining, capsulated bacilli, few streptococci.

Cultures on blood serum and sugar agar showed bacilli of the colon group.

Fibrin: Sections were stained by Gram's method and Aurantia, thionin, methylene blue and eosin, and Gram's method, methylene blue and eosin. Fibrillæ, leucocytes, plasma, and endothelial cells. Marked fragmentation. Many large Gram-staining bacilli morphologically like the B. aerogenes capsulatus of Welch. Several smaller non-Gram-staining bacilli. Plate XXIII., Figs. I. and II.

CASE V. — Woman, aged 30. Operation, laparotomy. In the mesentery of the small intestine several enlarged glands were found, varying in size from a chestnut to a large hazelnut. Microscopic examination of a portion of one of these glands showed tuberculosis. Several of these glands were shelled out of the mesentery. The abdominal wound was closed without drainage. Symptoms of peritonitis began on the second day after operation. Death on the seventh day.

Autopsy: General fibrino-purulent peritonitis. Tuberculosis of the mesenteric glands. The abdominal cavity contains a considerable amount of frank yellow pus. Intestinal coils dark red, injected, and glued together by a yellow fibrino-purulent material.

Smear preparation: Leucocytes. Several short chains of streptococci. Many small, non-Gram-staining bacilli with rounded ends.

Cultures on blood serum and sugar agar show streptococci and a bacillus of the colon group.

Fibrin: Fibrillæ well developed and filled with leucocytes and many plasma cells. At the edges of the section of the fibrin are many Gram-staining micrococci and a few non-Gram-staining bacilli. The distribution of the organisms in this case differs from other cases in that the bacteria lie on the surface of the fibrin, and are not contained within the meshes.

CASE VI. — Man, aged 49. Operation for partial intestinal obstruction. At the operation the duodenum was found dilated, with the small intestine collapsed below the lower end of the duodenum. The upper end of the jejunum was opened, and a stricture found. The stricture was relieved by a plastic operation, and the intestinal wound was closed with silk sutures. Symptoms of peritonitis appeared on the second day after operation. Pus appeared in the abdominal wound on the fourth day. Death on the eighth day.

Autopsy: Fibrinous peritonitis. Sutured wound at upper end of jejunum. Tuberculosis of lungs with cavity formation. The peritoneal cavity is moist, and the intestinal coils glued together by a grayish fibrinous exudate. The edges of the sutured wound in the jejunum are necrotic, and can easily be separated.

Smear preparation: Leucocytes. Non-Gram-staining bacilli and streptococci.

Cultures on blood serum and sugar agar show streptococci and a bacillus of the colon group.

Fibrin: Granular fibrillæ. Leucocytes. Considerable fragmentation. Few Gram-staining micrococci.

CASE VII. — Man, aged 39. *History:* Symptoms of appendicitis of ten days' duration. Operation, opening and draining a localized appendix abscess. The appendix was sloughed off at its proximal end. The wound was left open, and the abscess cavity drained. Cultures at operation showed a profuse growth of bacilli and a few streptococci. Pathological report: Gangrenous appendicitis. Death on the sixteenth day after operation.

Autopsy: General fibrino-purulent peritonitis. Peritoneal cavity contains a considerable amount of yellowish, semi-fluid, purulent material. The omentum is adherent to the intestine in places. In the meshes of the omentum and contained within adhesions are small pockets of yellow pus. Intestines in places glued together with fibrinous exudate. Some portions of the intestines are almost gangrenous. Appendix was found sloughed off.

Smear preparation: Leucocytes, streptococci, and a few, small, non-Gram-staining bacilli.

Cultures on blood serum and sugar agar showed streptococci and B. coli communis.

Fibrin: Fibrillæ, leucocytes, lymphocytes, and plasma cells. Many Gram-staining micrococci. Several small, non-Gram-staining bacilli. Cell elements few.

CASE VIII. — Woman, aged 63. *History:* Symptoms of chronic intestinal obstruction, with increasing symptoms for nine months. Three hours before operation the patient was seized with sharp abdominal pain, accompanied by vomiting. The lower part of the abdomen was distended. Operation, laparotomy. The abdominal cavity contained considerable free brownish fluid and fecal material. Intestines shiny, but injected. No fibrin on the intestines. A minute opening was found in the cecum two inches above the appendix. This opening was closed by a purse-string suture, and a Mixter tube was placed in the descending colon. The abdominal cavity was flushed out with salt solution. Pelvis and both flanks were drained. Death two hours after operation.

Autopsy: General fibrino-purulent peritonitis. Adeno-carcinoma of the transverse colon, with metastases of the liver. Large and deep ulcers on the ileum and cecum. The peritoneal cavity contains a moderate amount of putrid fluid, and the coils of the intestine are weakly adherent. In places there are patches of fibrino-purulent material.

Smear preparation: Leucocytes. Many bacilli of different diameters, the majority non-Gram-staining. Several large Gram-staining bacilli.

Cultures on blood serum and sugar agar showed B. coli communis.

Fibrin: Fibrillæ, many leucocytes. Several non-Gram-staining, small bacilli; few Gram-staining micrococci.

CASE IX. — Woman, aged 37. *History:* acute abdominal symptoms three days before death. Miscarriage on the second day. No operation.

Autopsy: General fibrino-purulent peritonitis. Pneumococcus septicemia. Peritoneal cavity contains a large amount of thick, yellowish, purulent material, supporting much shaggy fibrinous exudate. Intestines, liver, and spleen are almost completely covered with a thick, fibrinous exudate. Uterus and tubes show no evidence of acute infection. Cultures from heart blood show pure growth of pneumococci.

Smear preparation from peritoneal cavity: Many leucocytes. Several pneumococci free and in leucocytes.

Cultures on blood serum show pure growth of pneumococci.

Fibrin from small intestine and surface of spleen, stained by Gram's method, methylene blue, and eosin, show leucocytes, granular fibrillæ, and many pneumococci free, and contained within the leucocytes. Plate XXIV., Figs. 1 and 2.

CASE X. — Woman, aged 42. *History:* symptoms of acute abdominal infection eleven days before death. Leucocytosis varying between 42,000 and 53,000. No operation.

Autopsy: chronic purulent salpingitis. Diffuse fibrino-purulent peritonitis. Streptococcus septicemia. Peritoneal cavity contains 300 cc. of opaque, creamy-white pus. Adherent to the serosa of the small intestine are patches of grayish-white elastic membrane which is easily removable. There are also fibrinous masses on the surface of the liver. Right Fallopian tube thickened and contained greenish-yellow pus.

Smear preparation of abdominal pus showed leucocytes and very numerous streptococci.

Culture on blood serum showed a pure growth of streptococci.

Fibrin: Leucocytes, lymphocytes, and many plasma cells. Fibrillæ distinct. Many micrococci and streptococci.

CASE XI. — Man, aged 18. *History:* typhoid fever symptoms; Widal positive eight days before operation. Ten hours before operation, sharp abdominal pain followed by falling temperature with increasing leucocytosis to 14,000. Operation, laparotomy. The lower third of the abdomen was found filled with a milky fluid. The opacity of the fluid was greater in the pelvis. A few small flakes of fibrin were found on the surface of the small intestine, and were removed. A perforation of the small intestine was found fourteen inches above the ileo-cecal valve. The perforation was closed with a Lembert suture of silk, and the abdominal cavity washed out with salt solution. Abdominal wound closed with drainage. Recovery.

Smear preparation: Leucocytes. No bacteria.

Cultures on blood serum showed a few colonies of bacilli of the colon group. Cultures on sugar agar produced gas.

Fibrin: Few fibrillæ. Many leucocytes and lymphocytes. No bacteria. These sections were frozen sections and were stained with carbol-thionin.

CASE XII. — Man, aged 21. *History:* Typhoid fever of nineteen days' duration. Symptoms of perforation five hours before operation. Laparotomy. On opening the peritoneal cavity thin milky fluid and gas escaped. There was a fibrinous exudate on the lower part of the ileum; nine inches above the ileo-cecal valve is a perforation one-fourth inch in diameter. The perforation was closed with a silk Lembert suture. Laparotomy wound closed with drainage. Death on the third day after operation. Culture taken at the operation shows numerous colonies of Staphylococcus pyogenes albus and B. coli communis.

Autopsy: General fibrino-purulent peritonitis. Multiple typhoid ulcers of ileum with two perforations. The typhoid bacillus was isolated from the spleen. The abdominal cavity contained a moderate amount of yellow, semi-purulent material. The surface of the small intestine is covered with a yellowish scummy exudate. There are also fibrinous patches on the liver and spleen. The sutured perforation had broken away at the upper end of the opening. There were also two other perforations which were not sutured.

Smear preparation: Leucocytes, streptococci, and non-Gram-staining bacilli.

Cultures on blood serum and sugar agar showed bacilli of the colon group.

Fibrin: Fibrillæ distinct. Leucocytes. The majority of the organisms are streptococci, some of which are within the cells. There are also a few short, non-Gram-staining bacilli. Plate XXIII., Fig. 3.

CASE XIII. — Woman, aged 21. *History:* Miscarriage nine days before operation. Foul brownish vaginal discharge. Operation, curetting uterus. Death on the following day.

Autopsy: Abscess of pelvis. Diffuse fibrino-purulent peritonitis. Recent abortion. The abdominal cavity contains two quarts of thin, yellow purulent fluid. The loops of the small intestine are glued together with a fibrino-purulent material. The abscess cavity in the pelvis communicates by an opening with the rectum.

Smear preparation from peritoneal exudate: Mass of bacteria of very great variety.

Culture on blood serum and sugar agar showed B. coli communis.

Fibrin; Hyalin fibrillæ distinct. Many leucocytes. Many Gram-staining micrococci. Several medium-sized, rather thick bacilli with rounded ends, Gram-staining.

CASE XIV. — Woman, aged 52. *History:* Operation for cancer of uterus. Complete hysterectomy. Drainage through vaginal and abdominal wounds. Amputation of right leg for thrombo-phlebitis of right iliac vein. Death on the fifth day after laparotomy.

Autopsy: Peritoneal cavity contains a moderate amount of yellow, semi-purulent material, most marked in right iliac fossa. The coils of intestine are glued together at their margins of contact with a soft, purulent exudate. The serosa of the intestine shows considerable injection. In the posterior wall of the upper part of the rectum is an opening one cubic centimeter in diameter. The adjacent tissues are pus-laden, and these tissues are in direct relation with the pus-laden tissues in the right iliac fossa.

Smear preparation: Many leucocytes. Several small, non-Gram-staining bacilli, resembling the colon bacillus. Several slightly larger capsulated non-Gram-staining bacilli.

Cultures on blood serum and sugar agar showed B. coli communis and B. mucosus capsulatus.

Fibrin: Very few, small, non-Gram-staining bacilli. Leucocytes, lymphocytes, and plasma cells. Fibrillæ distinct. There is considerable fragmentation.

CASE XV. — Man, aged 42. *History:* Failing compensation of heart. Mitral stenosis. Acute abdominal symptoms two days before death

Autopsy: General peritonitis; icterus; streptococcus septicemia. Peritoneal cavity contains a large amount of opaque yellow fluid supporting yellowish flocculi. Serosa is injected. There are flakes of fibrin on the intestine, liver, and spleen.

Smear preparation: Leucocytes, lymphocytes, and several endothelial cells. Many streptococci.

Cultures on blood serum showed a pure growth of streptococci.

Fibrin: Many leucocytes; several endothelial and plasma cells. There are also several degenerated cells which are not inconsistent with degenerated lymphocytes. Few micrococci and streptococci.

CASE XVI. — Woman, aged 52. Operation, partial gastrectomy followed by end-to-end suture; drainage by wicks above and below the line of suture. Symptoms of peritonitis appeared on the fourth day after operation. Death on the eighth day.

Autopsy: General peritonitis. Open wound of stomach. Peritoneal cavity contains a moderate amount of brownish opaque fluid. There is a yellowish membranous exudate on the intestines of the upper part of the abdomen.

Smear preparation: Many leucocytes; few lymphocytes. Several large, capsulated, Gram-staining bacilli. These are regarded as B. aerogenes capsulatus. Many micrococci, Gram-staining; some in chains.

Cultures on blood serum showed streptococci, and an unknown, medium-sized, non-Gram-staining bacillus.

Fibrin: Few fibrillæ, many leucocytes, lymphocytes, and plasma cells. Several streptococci and several large, Gram-staining bacilli. Many non-Gram-staining bacilli resembling the unknown bacillus seen in the culture.

CASE XVII. —*History:* Acute abdominal symptoms eight days before death. No operation.

Autopsy: Embolism of the superior mesentery artey, with infarction of the cecum, ileum, and ascending colon. General peritonitis. Peritoneal cavity contains a small amount of opaque fluid, supporting greenish-yellow flocculi. Intestines are injected, and at the margins of contact present streaks of greenish-yellow fibrinous exudate.

Smear preparation: Many leucocytes, lymphocytes, and several endothelial cells. Many capsulated, non-Gram-staining bacilli of varying sizes. Few medium-sized Gram-staining bacilli and micrococci.

Cultures on blood serum and sugar agar showed B. coli communis and B. mucosus capsulatus.

Fibrin: Fibrillæ distinct. Leucocytes, lymphocytes, and endothelial cells. Some fragmentation. Many small, non-Gram-staining bacilli of varying sizes. Few slightly larger Gram-staining bacilli and a few short chains of streptococci.

CASE XVIII. — *History:* Intestinal obstruction from cancer of the transverse colon. Operation, resection of transverse colon, end-to-end suture drainage. On the third day after the operation the abdominal cavity was reopened. Purulent fluid escaped. The intestines were injected, and covered with fibrinous flakes. Patient died three hours after second operation. No autopsy.

Smear preparation: Leucocytes, non-Gram-staining bacilli and several streptococci.

Cultures on blood serum and sugar agar showed a profuse growth of bacilli of the colon group and streptococci.

Fibrin: Much granular material; small fibrillæ poorly developed. Many leucocytes and lymphocytes. Many micrococci, some in chains. Several non-Gram-staining bacilli of varying diameters and lengths.

CASE XIX. — Woman, aged 40. *History:* Intestinal obstruction of five days' duration. The obstruction was in the pelvis, and due to a pelvic abscess and adhesions. Operation, inguinal colotomy, with drainage of the pelvis. Culture taken at operation showed the B. mucosus capsulatus. Death on the fifth day.

Autopsy: Pelvic abscess; general peritonitis. Very small amount of free fluid in the peritoneal cavity. One coil of intestine, dark gray in color, with dark red streaks along the margins of the contiguous coils.

Smear preparation: Streptococci and several non-Gram-staining capsulated bacilli.

Cultures showed streptococci and the colon bacillus.

Fibrin: Granular fibrillæ, leucocytes, lymphocytes, and several plasma cells. Numerous micrococci and bacilli at the edges on the surface of the fibrin.

Summary.

Material was collected from nineteen cases of acute peritonitis. In seventeen of these cases bacteria were found in the fibrin. In two cases organisms were present in cover glass and culture, but could not be identified in the fibrin.

In three cases (Nos. IX., X., and XV.) no lesion was found in the peritoneal cavity as a cause for the peritonitis. In one of these, Case IX., pneumococci were present in smear preparation, in culture, and in the fibrin. The duration of the infection in this case was three days.

In Cases X. and XV. streptococci alone were found in the smear preparation, culture, and fibrin. The duration of the disease in Case X. was eleven days and in Case XV., two days.

In seven cases (Nos. I., V., VI., VIII., XII., XVI., and XVIII.) streptococci and colon bacilli were present in the smear preparations, culture, and fibrin. In some of these cases, however, one of the organisms occasionally failed to be isolated in culture, although the organism was present in the smear and in the fibrin.

Two cases (Nos. I. and IV.) showed streptococci, colon bacilli, and B. aerogenes capsulatus. Bacillus aerogenes capsulatus was present in the smear preparation and in the fibrin in each case, but was not isolated in culture, although anaerobic cultures were made.

Case XIX. showed streptococci and colon bacilli in the smear preparation, in the culture, and in the fibrin. A culture in this case was taken at the operation five days before death, and showed streptococci and the B. mucosus capsulatus.

Case II. showed colon bacilli in smear preparation, in the culture, and in the fibrin. Case XVIII. showed in the smear preparation and in the fibrin B. mucosus capsulatus, colon bacilli, and B. aerogenes capsulatus. Culture in this case showed colon bacilli and B. mucosus capsulatus.

In Case III. the colon bacilli and B. aerogenes capsulatus were present in smear preparation and in the fibrin. Culture showed the colon bacilli only.

Case XIV. showed the colon bacilli and B. mucosus capsulatus in the smear preparation and culture, while the colon bacillus alone was present in the fibrin.

It is to be concluded from the observations in these nineteen cases that in most cases of acute peritonitis with fibrinous exudation bacteria are present in large numbers in the fibrin.

(I am greatly indebted to Dr. J. H. Wright for his encouragement and advice in carrying out this work. I also wish to thank the staff of the Massachusetts General Hospital for their kindness in allowing me to use their material.)

DESCRIPTION OF PLATES.

PLATE XXIII.

Fig. 1. Case IV. Smear preparation of abdominal pus, showing bacillus aerogenes capsulatus and bacillus mucosus capsulatus. Stained by Gram's method, eosin and methylene blue.

Fig. 2. Case IV. Section of fibrinous exudate, showing leucocytes and the bacillus aerogenes capsulatus in the meshwork of fibrin. Stained by Gram's method, eosin and methylene blue.

Fig. 3. Case XI. Section of fibrinous exudate, showing masses of streptococci, fibrin, and leucocytes. Stained by Gram's method and thionin.

Fig. 4. Case XV. Smear preparation of pus in the abdominal cavity, showing streptococci and degenerated leucocytes stained by Gram's method, eosin and methylene blue.

Fig. 5. Case XV. Section of fibrinous exudate, showing streptococci, fibrin, and leucocytes. Stained by Gram's method and Aurantia.

PLATE XXIV.

Fig. 1. Case IX. Smear preparation of pus in the abdominal cavity, showing pneumococci free and within the leucocytes. Stained by Gram's method, eosin and methylene blue.

Fig. 2. Case IX. Section of the fibrinous exudate, showing pneumococci, fibrin, and degenerated leucocytes. Section stained by Gram's method and Aurantia.

(The photographs were made by Mr. L. S. Brown in the Clinico-Pathological Laboratory of the Massachusetts General Hospital.)

TRANSPLANTATION OF ARTERIES.

JOSHUA C. HUBBARD, M.D.

(For the Division of Surgery of the Medical School of Harvard University.)

Until recently the surgery of arteries has been confined to the repair of tears in the vessel wall. In those cases where the tear is of small extent the defect is closed by sutures. When the tear extends more than one-half across the vessel it has been shown by Murphy (N.Y. Med. Record, Jan. 16, 1897) that simple suturing is unsatisfactory, because by so extensive a wound of the wall many of the vasa vasorum are destroyed. As a result, the nutrition of the wall is so reduced that it eventually sloughs along the suture line. To repair an artery, when so severely torn, resort must be had to either Murphy's invagination method (N.Y. Med. Record, Jan. 16, 1897) or to the magnesium cylinders of Payr (Arch. f. Klin. Chir., 1900, H1).

Fine silk has been found to be the best suture material (Doerfler, Beitr. z. klin. Chir., 1900) of such a size that it will fill as nearly as possible the needle holes in the vessel wall. The needles are to be the finest which can be handled. Doerfler (Beitr. z. klin. Chir., 1900) proved experimentally that sutures which appear in the lumen of the vessel do not act as foreign bodies and cause thrombi. He advocated, therefore, passing the sutures through all the layers, because more practical than including only the adventitia and media. Jacobsthal, who examined some of Doerfler's specimens microscopically, found that in a few days a thickening of the endothelium occurs, which surrounds the stitches and thus shuts them off from the lumen of the vessel.

The problem before me in this experimental work has been the radical cure by surgical means of aneurisms of the abdominal aorta. As many abdominal aneurisms have a branch to some part of the alimentary tract coming from the sac, the first step in the cure of the aneurism proper must concern itself with the treatment of this branch. One of the larger branches to the intestine cannot be tied off, for by so doing the circulation to that portion of the intestine is suddenly changed. We have learned from cases of thrombosis of the mesenteric arteries that the branches going to the intestine are practically end arteries, and that the sudden stopping of the circulation in one of them results in gangrene of that portion of the intestine supplied by it. The problem then is to deal with this branch coming from the sac, in such a manner that it will permanently continue to supply blood to its portion of the intestine, or will be shut off so slowly that time will be given for the establishment of a collateral circulation. Transplantation of this branch to some neighboring part of the aorta will fulfill these requirements, if the anastomosis will remain patent permanently or for a sufficient length of time. My work was thus reduced to establishing first the practicability of transplanting arteries. All my experiments were done on dogs. The carotids were chosen as the vessels to be transplanted because of their accessibility and size.

From the work already done by Murphy it was evident that it was useless to sew the end of a divided vessel into a hole in another because of the sloughing along the suture line from the destruction of the vasa vasorum. In order to minimize this destruction as much as possible, I began by doing a lateral anastomosis, the technic of which was as follows: The dog was given a subcutaneous injection of morphine, and then etherized. The skin of the neck was cleaned by shaving and washing with corrosive. Each carotid was isolated, and one divided between ligatures at the base of the neck. The divided vessel was pulled across the neck, and the point decided upon where the anastomosis was to be made. To control the circulation during the operation a strip of gauze was passed around the divided vessel some distance from the end and two others around the intact carotid, one above and one below the point chosen for the joint. These bands were pulled tight enough to control the circulation and held by hemostats. A narrow strip was excised from the wall of each vessel, and the corresponding edges of the slits thus made were sewed together by No. 4 silk on fine curved needles, the sutures including all the layers. After finishing the suturing, the joint was tested by releasing the distal gauzes first. Any oozing was controlled by another stitch or two or by light pressure for a moment. By then loosening the proximal controlling gauze strip, the full blood pressure was allowed to come on the anastomosis.

Experiment I. — At end of operation doubtful whether pulsations felt in the transplanted artery. Dog died on fifth day. The carotid found shut off proximal to the anastomosis by a stitch too deeply placed. The anastomosis patent, as shown by the passing of air from a blow pipe, through the anastomosis, from the transplanted vessel and out the distal end of the carotid.

Experiment II. — At end of operation blood passed through anastomosis. Dog etherized nineteen days later. Vessels dissected out and pulsations almost as strong in the transplanted vessel as in the main artery. On section vessel walls very much thickened at the site of the anastomosis and the lumen lessened. Portion of a silk stitch free in the lumen; slight evidence in the wall of another suture, otherwise none seen from the inside. The connection between the vessels was through several small holes.

Experiment III. — At end of operation blood passed freely through anastomosis. When animal killed, about one month later, vessels thrombosed.

Experiment IV. — At end of operation doubtful whether blood passed through anastomosis. When killed later, vessels thrombosed.

Experiment V. — After ligating the carotid, it was not divided, but drawn across the neck and anastomosed to the other. At end of operation the blood passed through the anastomosis. When killed two days later thrombus at site of anastomosis.

Thinking that perhaps the anastomosis which was found patent at the end of nineteen days might have been done unconsciously in such a way that intima was brought in contact with intima, I then changed my technic. A slit was made in each vessel, and sutures were so placed that intima was brought in contact with intima.

Experiment I. — At the end of the operation blood passed through the anastomosis. When animal killed three days later, clot found at the site of the anastomosis.

Experiment II. — At the end of operation blood passed through the anastomosis. When animal killed two days later, thrombus found at the anastomosis and at the site of one of the controlling gauzes.

EXPERIMENT III. — No. 1 silk and fine cambric needles used. At end of operation blood passed through the anastomosis. When animal killed four days later, air could be blown through the main vessel, but not through the anastomosis, which was obstructed by a clot.

I then changed the technic entirely, and made an anastomosis in the following manner. Both carotids were isolated. One was tied low in the neck. A controlling gauze band was placed about it, high up in the neck. One vessel was divided just above the tie. The end of the vessel was split, so that two flaps were made. Controlling gauzes were placed around the main carotid. A wedge-shaped piece was cut from its wall. The lumen of the divided vessel was placed over the hole, and the two flaps were carried around the undivided carotid and sutured to each other and the sheath of the vessel. This part of the operation was done several times before it was satisfactory. The first two times the small size of the hole prevented a free flow of blood into the transplanted vessel. At the third attempt the hole was enlarged, and the blood passed through feebly. A stitch was required at the angle of the flaps to prevent oozing. Two days later the animal was etherized, and the vessels cut down upon. The condition was then the same as at the end of the operation, the pulsations being strong in the main vessel beyond the anastomosis and feeble in the transplanted one. Fifteen days after the first operation the animal was killed. Air from a blow-pipe passed through the anastomosis and out of the end of the transplanted vessel, but before it came through the main vessel a small, soft, dark clot was forced out.

I have not been able to further test this method from lack of material, but it seems superior to the others in that it minimizes the handling of the vessels, and the bruising of the intima causes, for thrombus formation, and that it can be done with few sutures, perhaps all being outside the lumina. Care must be taken that the hole is large, and that the flaps do not act as ligatures. A criticism which might justly be made of the earlier methods is, that later the line of the suture might weaken and the vessel wall give way. By this method there is no fear of this, as the wall is stronger after the operation than before.

In all the above experiments it was possible to make an anastomosis which would not leak, no matter what method was used. In seven of these nine experiments an anastomosis was made which transmitted the circulation satisfactorily for the time being. In one the anastomosis was patent five days after the operation, though the main vessel was thrombosed, and in another, where the result was perfect two days after operation, at fifteen days after operation a small and apparently recent clot was found in the main vessel, though the anastomosis itself was functionary, and in a third there was perfect success nineteen days after operation, when the dog was killed. When it is realized that the vessels with which I have been working are about the size of the radial artery in man, and even smaller when the circulation is controlled, it will be appreciated that the mechanical difficulties of placing the sutures without injuring the intima are great. If results can be obtained under these circumstances which are at all satisfactory, how much more hopeful is the outlook when the vessels are of large size.

All of the above experiments were carried out on the carotids. The technic of transplanting a branch of the aorta would not differ. The cord would be deprived temporarily of its blood supply, and the result of this might be questioned. In two animals I attempted to transplant branches of the aorta, and controlled the circulation long enough to perform the operation. Neither of the cases was successful as far as the anastomosis was concerned, the first because the transplanted branch was too small to work on, and the second because it was under so much tension in its new position that during the muscular spasms of death from a subcutaneous injection of strychnia it was torn away from the aorta. In neither case, however, did the animal show any ill effects from the temporary anemia of the cord.

Doctor Matas (Ann. of Surgery, Feb., 1903) has already published a report of three cases of aneurism cured by suturing the sac, leaving a lumen when possible. This portion of my original problem has, therefore, been already solved. By combining his technic with mine, it seems that it should be possible to cure an aneurism of the abdominal aorta. The aneurism can be sutured by the method of Matas, preserving its lumen, and any branches which arise from its sac and are too important to obliterate can be transferred to another portion of the aorta.[1]

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[1] I should hesitate to present such a paper as this, which leaves so many points unsettled, were it not that this is the first report by the Committee on Surgical Research, to whom I am indebted for my opportunity to experiment. As I hope some time in the future to continue this work, I make my offering now simply as a preliminary report.

DESCRIPTION OF PLATE XXV.

Fig. 1. Scheme for carotid transplantation.

Fig. 2. Cross-section of vessels at anastomosis, showing the vessel walls turned, bringing intima against intima. A. intima.

Figs. 3–4. Last method of anastomosis described.

THE EFFECT OF X-RAYS ON LIVING TISSUE.[1]

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(*For the Division of Surgery of the Medical School of Harvard University.*)

From the conflicting statements about the effects of X-rays in therapeutic work, experiments have been made on various sorts of living tissues, in the hope that if definite pathological results could not be found in human tissues, one might reason by analogy from experiments along other lines. In attempting to eliminate errors from the results there arose so many problems in electricity, physics, biology, and pathology as to make it almost impossible to come to accurate conclusions, so that this report is made rather as a preliminary than as a final one.

With the exception of the cases treated, and unless otherwise stated, the method of exposure to X-rays was to have the object on an insulated support as nearly as possible in the line of the angle of reflection of the cathode stream, so that the greatest amount of X-rays were received. Between the tube and object was a grounded aluminum screen, the wire to the ground and the two feed wires to the tube being as far from the object as possible, so that the magnetic field about the tube was reduced to a minimum. Electroscopic tests on the distal side of the grounded screen were negative.

The apparatus used was usually a twelve-inch Heinze coil, which is a high potential, but not a high frequency, machine, and the quality of light was such as to show clearly the bones of the wrist. Changes of air, temperature, light, and moisture were guarded against as far as might be.

The material worked with included vinegar worms (angelula aceti), protozoa, capillaries of frogs, etc., frogs' hearts, tortoise hearts, beans, lupin seeds, human leucocytes, clinical cases of cancer and tuberculosis, and "burned" human skin.

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[1] Only through the kindness of Mr. Walter J. Dodd, of the Massachusetts General Hospital, have I been able to do a large part of this work. I have also to thank him for clinical material and for much valuable advice and assistance.

VINEGAR WORMS.

1. Several drops of vinegar containing numerous worms were exposed for thirty-six minutes at forty-five centimeters' distance from the anode, and observed under a low-power microscope during the whole time. One-half hour after exposure the majority of the worms were dead, the others being coiled up and making irregular movements. Two controls unchanged.

2. Similar experiment repeated at the same distance for eighteen minutes. Result in half an hour, many worms dead or dying, but not so many as before. Control unchanged.

3. Experiment repeated with a Kinraide high-frequency coil. Result in half an hour only a few worms dead. Control unchanged.

4. Same experiment without grounded screen; twenty-one minutes' exposure. Result same as in No. 1. Controls unchanged.

5. Two similar amounts of vinegar taken. (*a*) Exposed to X-rays without grounded screen nineteen minutes, ten centimeters from anode. (*b*) Similarly exposed, but protected from X-rays by sheet lead, being in the magnetic field. Result: (*a*) worms nearly all killed. (*b*) Between one-third and one-half killed. Examined immediately after exposure.

6. At forty centimeters' distance for one and one-half minutes; no result.

7. At forty centimeters from the anode, "low" penetrating light, bones of the hand being faintly seen. Exposure thirty minutes, no change seen.

From these experiments it appears that long exposures were necessary to affect the worms; that less effect was obtained from the high frequency apparatus; that X-rays together with an active magnetic field were more fatal than the magnetic field alone, and that a highly penetrating light was more harmful than a low one.

PROTOZOA.

A two-weeks' old infusion of hay showed under the microscope many rapidly swimming protozoa, each surrounded with a membrane and containing moving protoplasm and a nucleus. Groups of protozoa were frequently seen feeding on clumps of dirt or chains of bacteria.

1. Exposed forty-five minutes at ten centimeters from anode and observed under microscope during whole time. After the first five minutes the protozoa were no longer seen to feed in clusters, but became separated with possibly a livelier motion. At end of exposure a few were motionless.

One and a half hours later nearly all were dead; a few were swimming slowly. Control unchanged until the water dried up.

2. Similar experiment, similar result in thirty minutes.

3, 4, and 5. With exposures of varying duration under thirty minutes no appreciable effect, except the failure to feed in groups after the first few minutes.

6. Exposure to high frequency brush discharge for thirty seconds killed all individuals. Control unchanged.

CAPILLARY CIRCULATION.

1. The hind feet of a chloroformed frog were pinned out so that the capillary circulation in the web could be seen under the microscope, one microscope being used for each foot. Foot (*a*) exposed to X-rays sixteen minutes at fifteen centimeters' distance. Foot (*b*) protected from X-rays by sheet lead. Both feet otherwise under the same conditions. At end of exposure the capillaries in web (*a*) showed dilatation to nearly double the size of web (*b*). (There seemed to be a slight dilatation in web (*b*), probably from the irritation of the pins and stretched condition of the web.) No emigration of leucocytes could be seen. Fifteen minutes later the capillaries had almost regained their former size; the light was again turned on and the capillaries again dilated.

2. The same frog twenty-four hours later; same experiment repeated, using the previously protected foot for

exposure. Same effect noticed in fifteen minutes. Control unchanged.

3, 4, and 5. Attempts were made to perform similar experiments on three tadpoles, two white mice, and one guinea-pig, in each case chloroform being used; but owing to the impossibility of keeping the animals quiet, the field under the microscope could not be observed throughout, though it was thought that the same result appeared. In the guinea-pigs' mesentery a marked dilatation was seen in three minutes. But as the capillaries in the peritoneum dilate anyway when the abdomen is opened, one can conclude nothing from this.

FROGS' HEARTS.

1. A frog's heart was excised and kept moist with normal salt solution, beating eighteen to the minute, and exposed to X-rays for eleven minutes at twenty-five centimeters' distance. At end of nine minutes the heart's rate was still eighteen. At end of ten and a half minutes only a faint contraction could be seen; in eleven minutes no contraction; no recovery in one hour.

2. Two frogs used. Frog (*a*) killed by electrocution; frog (*b*) by chloroform. Hearts of both excised and kept moist with salt solution, heart (*a*) beating strongly at thirty-two to the minute, heart (*b*) beating weakly at twenty-eight. After an exposure to X-rays of one minute and ten seconds at twenty-five centimeters' distance from the anode, heart (*a*) had ceased beating. After exposure at same distance for one minute, heart (*b*) had ceased beating. No recovery of either heart in half an hour.

KYMOGRAPHIC TRACINGS OF HEART ACTION IN LIVING ANIMALS.

Seven frogs and one tortoise were used. After pithing the brain and spinal cord so that the animal would keep still, the thorax and pericardium were opened and the heart made to write kymographic tracings in the ordinary way. Twelve records in all were made. Owing to the kymograph being small and the X-rays not immediate in their action it was not easy to show the effect in one revolution of the drum, unless it ran so slowly that the excursions of the writing point overlapped. The entire animal was protected from the X-rays by sheet lead, except for a window which just allowed the auricles and ventricles to receive the light, so that whatever effect was obtained must have been solely on the heart. Varying lengths of exposures were made between five and thirty minutes; in no case was there any effect with less than three minutes' exposure, and usually there was none under ten minutes. In all seventeen tracings were made, four of which showed no change. Of the other thirteen all showed a shorter excursion of the writing point, and five of the thirteen showed both a shorter and a slower excursion, after the X-rays were turned on. The failures were in fresh, strongly beating hearts, and the inhibitory effect was best seen when the hearts were either tired or overloaded. (See Plate XXVI., 1.) The tracing reproduced here was made by a tortoise's heart. In this case the writing point was an electric spark arranged to make a record without friction. The smaller frogs, especially the females, were found unsatisfactory. Professors William T. Porter and W. B. Cannon have been kind enough to point out one or two possible errors in these experiments, such, for instance, as the lack of a control frog and the failure to be sure that the temperature of the air did not vary during the experiments, but it would seem as if these results, taken in connection with those of the excised hearts, were at least suggestive of a direct inhibitory effect.

BEANS.

Twenty-eight beans (Faba vulgaris) were planted in two flower-pots. The first pot received in all one hour and forty minutes of X-rays, as near to the pot as the growth would allow. The second pot received no X-rays. The beans in the X-rayed pot appeared above the earth first and averaged a greater height than those in the other pot. Thirteen beans grew up in the X-rayed pot and seven only in the control.

To obviate any error in the previous experiments, especially with regard to the electric field, seven beans were planted in each half of the same pot, and the control side protected with one-quarter inch sheet lead during the exposure. X-rays were given for ten minutes daily for seven days, a total of one hour and ten minutes. In three weeks the conditions were as shown in the picture. (See Plate XXVI.)

Similar results were obtained with two other pots of beans, one pot of nasturtiums, one of lupins, and one of mustard, though the difference between those exposed to X-rays and the controls was not so marked as in the more rapidly growing beans. In all cases the plants that received the X-rays did not survive so long as those not receiving the rays, though the former appeared above the earth first and grew to a greater height. They appeared to be over-stimulated. In all cases the plant received the same amount of water (measured) and daylight.

To see what effect the X-rays would have on a full-grown plant, some weeds were picked in a swamp early in spring. After an exposure of ten minutes daily for ten days within ten centimeters of the anode, no variation from the control could be seen. A similar failure was found with willow-buds.

HUMAN LEUCOCYTES.

In seven individuals white and red blood counts were made, three slides of leucocytes being counted in each case. Then X-ray exposures were given for ten minutes in the ordinary way and the blood again counted immediately. In three of the cases the first count was made from one ear, then the exposure made on the other ear, and the second count immediately taken from the exposed ear. In one the exposure was on the forearm, and in three on the face; in these cases the second count was taken from a point at least two inches distant from the place where the first had been taken, in order to avoid any leucocytosis from the irritation of the first prick.

Results: No variation was found in the red cells.
Leucocytes:

CASE 1. — Face.	Before exposure . . .	5,000
	After " . . .	9,600
CASE 2. — Arm.	Immediately after dinner.	
	Before exposure . . .	11,200
	After " . . .	13,600
CASE 3. — Ear.	Before " . . .	7,000
	After " . . .	6,600
CASE 4. — Face.	Before " . . .	6,000
	After " . . .	8,200
CASE 5. — Cheek.	Before " . . .	6,500
	After " . . .	9,200
CASE 6. — Ear.	Before " . . .	6,800
	After " . . .	8,140
CASE 7. — Ear.	Before " . . .	6,800
	After " . . .	11,500

Cases 2 and 3 were healthy individuals exposed for experiment only. All the other cases were exposed for existing malignant or tubercular disease. In one case (3) there was no increase in the number of white corpuscles, and in the other six an average increase of nearly three thousand. If these results can be substantiated later, they will at least suggest that the presence of an increased number of leucocytes in tissues exposed to the X-rays has a bearing upon their curative action in superficial lesions. Whether or not the cure is due to the phagocytic action of the leucocytes can be only a matter of conjecture.

PATHOLOGICAL SECTIONS.[1]

In all, eighteen cases have been studied clinically and histologically: nine of epithelioma of the face, all of which healed from a clinical point of visio; one of carcinoma of the cheek, not healed; three of carcinoma mammæ, none healed; one of scrofuloderma, healed; one of lupus vulgaris, healed; two X-ray burns of three years' standing, healed after excision of the burned skin; and one case of frog skin exposed fifty-seven minutes.

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[1] My thanks are due to Dr. E. H. Nichols, Dr. Walter C. Howe, and Dr. Channing C. Simmons for valuable assistance in studying the microscopic slides. Also to Dr. Simmons for the use of one of his cases.

NINE CASES OF EPITHELIOMA OF THE FACE, ALL OF AT LEAST TWO YEARS' STANDING, HISTOLOGICAL DIAGNOSES HAVING BEEN MADE BEFORE BEGINNING TREATMENT.

The sections studied were all taken after sufficient exposure to X-rays to have produced a slight dermatitis and a partial healing, and were excised either from a healed area or from the margin of the ulcer that was in process of healing. All of the lesions from which the sections were excised healed up later and have remained healed for a year. In other words, the cancers were disappearing at the time the sections were excised.

This case will serve as a type of this group. (See Plate XXVII., 1 and 2.) Diagnosis of epidermoid cancer was made histologically. Duration of disease seven years. After ninety minutes of exposure during two months, my notes say, "Scab almost gone. Given final exposure of seven minutes at three and a half inches from anode. Specimen taken from healed area in front of nose, including nodule." (See Plate XXVII., 3.)

This specimen shows "a typical rodent ulcer entirely covered by epithelial cells which dip down in places. Necrosis and hyaline degeneration occasionally." [1]

Here, then, a bridging of the epidermis has taken place over a typically malignant nodule, which had been unhealed up to the time the X-rays were given. The areas of degeneration and necrosis after exposure and skinning over of the epidermis are no more than might be found in any such growth, whether treated by X-rays or not; in other words, there is no appreciable effect on the cells microscopically, although healing apparently took place over them.

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[1] Report of Dr. Howe.

In contrast to the superficial lesions above described, the following sections from deeper lesions were examined: From a large rapidly-growing, squamous-cell carcinoma of the face and neck a specimen was taken from the subcutaneous tissue early in the treatment. This specimen shows "many epithelial pearls, some containing leucocytes; new connective tissue, hemorrhage in small areas, deeper masses of epithelial cells showing necrosis and softening. In places the epithelial cells are perfectly preserved. Cells of the sebaceous glands normal. A certain amount of endarteritis. Two mitoses seen."[1]

After thirty X-ray exposures by Mr. Dodd, causing considerable dermatitis and a slight macroscopic improvement, a piece was excised from the same area as before. "Included masses of epithelium are more numerous. Epithelial pearls show degeneration and contain leucocytes. Many lymphoid and plasma cells. Hemorrhage on surface of section. Mitoses rare."[2] (Plate XXVIII., 1 and 2.)

"None of these specimens show any satisfactory evidence from a microscopic standpoint that the carcinoma cells are in extensive process of resorption."[3]

In other words, there are no changes in the microscopic elements to be shown after thirty treatments by X-rays, although clinically the case was slightly improved.

This case became rapidly worse and disappeared. He is doubtless dead.

Tumor of breast. "Narrow columns of epithelial cells with a relatively large amount of connective tissue stroma. Scirrhus cancer."[3] After sixty-seven X-ray exposures a section was removed from the same area as before. "The skin was slightly thickened and everywhere deeply reddened with superficial, cicatricial contraction. Microscopic examination shows it to be made up of a plexus of quite small epithelial cells separated by relatively dense bands of connective tissue. The cells in general were small and slightly irregular, but there were numerous mitoses present."[1] (Plate XXVIII., 3 and 4.)

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[1] Report of Dr. E. H. Nichols.

[2] Report of Dr. Howe.

[3] Report of Dr. J. H. Wright.

In other words, there were no microscopic changes, except on the skin, referable to the X-ray treatment, although the case clinically improved; in fact, the carcinoma had become more medullary, if anything. The patient is still living, two and a half years after beginning treatment.

An X-ray burn of three-years' standing, resulting from a single radiographic exposure in a neighboring town, was excised under ether. Microscopic examination: "Considerable increase of connective tissue of the corium and of subcutaneous tissue. In places there is infiltration of the corium and subcutaneous tissue with small cells. Some of the lymph channels of the corium are markedly dilated. Epidermis of one part of the section is lacking, and there is necrosis of the underlying tissue. The Malpighian layer of the epidermis shows marked irregularity in the outline of the inferior border and sends downward into the corium short processes."[2] (Plate XXVIII., 5.)

Here are evidences of destruction of the superficial tissues similar to what might be produced by any caustic, but in the deeper layers there was no microscopic change in the cellular elements nor were any macroscopic changes seen during the operation of excision. In other words, the destructive effect was seen only superficially.

The skin of a frog exposed fifty-seven minutes at fifteen centimeters' distance from anode and immediately hardened in Zenker's fluid, showed on section no variation from the control, except one dilated blood vessel containing leucocytes.

In several of the above specimens there are evidences of necrosis, endarteritis, increased formation of connective and elastic tissue, and dilated blood vessels and lymph channels. But these changes do not occur sufficiently often to draw conclusions from them. I cannot substantiate the statements made by certain writers[1] with regard to necrosis of deep tissues or alterations in deep malignant growths referable to X-ray treatment.

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[1] Report of Dr. William F. Whitney.

[2] Report of Dr. J. H. Wright.

Apparently, then, X-rays exert their action on the surface only; just as one's clothing or an aluminum screen will generally prevent a burn of the skin, so does the skin prevent a burn of the underlying structures. If it were possible to expose deep malignant growths without the intervention of any structure, very likely they would be cured as the surface ones are. While a burn is not necessary to produce healing in an epithelioma, a slight dermatitis hastens it. Of course, if necrosis is produced by an excessive exposure of the diseased tissues there will be both destruction of the tissue and the disease, but in my nine cases of epithelioma there was no more degeneration or starvation of the cellular elements than there might be in any cancer, yet the cases all healed; besides, the resulting scars were too pliable, and the tissue underneath too much filled out to have followed much necrosis. Evidently there is some action of the X-rays that is not a necrotic one, but which heals certain skin diseases and improves certain deep ones, but just what the cause of the healing is cannot be determined. Electrification may have something to do with it, as this factor cannot be entirely ruled out. On the other hand, it is well known that when an electrified body is passed through the path of X-rays it loses its charge, and it may be that the normal electrical action which always accompanies life is in some way similarly altered. But in the light of the above experiments a simpler hypothesis is that the irritant effect produced by the exposure brings more blood to the part and also gives a greater opportunity for phagocytosis. By the dilatation of the superficial capillaries the disappearance of pain may be explained on the grounds of diminished deep congestion caused by increased superficial congestion. This action, while being similar to that of any counter-irritant like iodine or a hot-water bag, is, however, usually more than temporary.

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[1] Sterne, Indiana Med. Journal, August, 1902. Grubbé, N. Y. Med. Rec., Nov. 1, 1902. Hopkins, Phila. Med. Journal, April 15, 1902, etc.

When we consider that a low penetrating light (poor in X-rays) is more effective than a high penetrating light (rich in X-rays), we must infer that the clinical effects are not due to X-rays so much as they are to some other unknown phenomenon. That this phenomenon is not simply the brush discharge is suggested by the fact that the most effective tube is the low one, from which the brush discharge is weak. Furthermore the same results are apparent, when the electrostatic effect is ruled out by a grounded screen. Discouraging as are the ued results on deep malignant growths, there is no doubt that with rare exceptions the cases treated are relieved of pain, gain in weight, improve in general condition, and live longer than they would have otherwise.

CONCLUSIONS.

1. The effect of prolonged X-ray exposures on vinegar worms and protozoa is fatal.

2. On superficial capillary circulation the effect is like that of an irritant, producing vaso-dilatation. The relief of pain in cases of deep malignant growths may be explained by this phenomenon.

3. On the heart in cold-blooded animals X-rays act as an inhibitor.

4. On growing plants the effect is distinctly stimulating, in fact, over-stimulating. On full-grown plants it is not apparently harmful.

5. Exposures produce immediately a local increase in the number of leucocytes in the blood. This is consistent with the irritant effect upon the skin and capillaries.

6. On surface malignant growths the effect is curative in a great majority of cases, the cure not necessarily being the result of necrosis.

7. On deep malignant growths there is no microscopic change to be found in the pathological or normal cell elements.

8. Excessive exposure on healthy skin produces sloughing of the superficial tissues, leaving a low-grade, sluggish

ulceration which heals slowly or not at all. Permanent dilatation of the capillaries and a scarcity of granulation tissue and blood vessels are common in such ulcerations.

9. Clinical effects are not due to X-rays so much as they are to some other unknown, but closely allied phenomenon.

EXPLANATION OF PLATES.

PLATE XXVI.

1. Tracing of tortoise heart. Record written on kymograph with electric spark, to avoid friction. X-rays turned on at first white line above tracing, turned off at second white line.

2. Seven beans planted in each half of pot. Right-hand half exposed seventy minutes to X-rays. Left-hand half protected during exposure by one-quarter inch sheet lead.

PLATE XXVII.

1. Epidermoid cancer of nose. Before treatment.

2. Same case as 1, after total exposure to X-rays of one hour and thirty minutes, during a period of two months.

3. Section of skin from "healed" area from case shown in 1 and 2.

4. Same specimen as 3, with high power.

PLATE XXVIII.

1. Squamous cell carcinoma of cheek. Section of tumor showing infiltration of subcutaneous tissue at beginning of treatment.

2. Same case as 1. Section from similar portion of tumor, showing no change in appearance after thirty X-ray exposures.

3. Scirrhus carcinoma of breast. Section of tumor showing columns of atypical epithelial cells.

4. Same case as 3, after sixty-seven X-ray exposures.

5. Section of margin of ulcer produced by X-ray burn of skin of three years' standing, showing changes only in superficial tissues.

ON THE INCREASE OF BACTERIOLYTIC COMPLEMENTS IN THE RABBIT'S BLOOD.*

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INTRODUCTION.

The instructive facts brought out by Sweet[1] in his study of a hemolytic complement for bovine erythrocytes in the rabbit's serum suggested the advisability of a similar inquiry into the peculiarities of the bacteriolytic complements. With this in view, an effort has been made to determine if the influences that favor an increase of hemolysis are correspondingly potent for an increase of bacteriolysis, and to discover if, as was shown for hemolysis, the increase in bacteriolytic activity is due to an increase in the amount of complement in the blood.

Among the results of his studies Sweet demonstrated, 1. That it is possible to increase the amount of hemolytic complement in the circulating blood by the induction of either septic or aseptic inflammatory reactions. 2. That this complement is probably not derived from any type of leucocyte. 3. That it is probably incapable of passing through animal membranes by dialysis.

In the course of the past few years numerous investigators have shown that by the injection into animals of a fairly wide range of both specific and non-specific substances it is possible to induce varying degrees of resistance to infection and intoxication.

Thus, for instance, M. G. Klemperer,[2] Lazarus,[3] and Metchnikoff[4] found that injections of serum protected guinea-pigs against intoxication. Pawlowsky[5] found that injection of proteids (papaiotin, alerin) that are positively chemotactic

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are capable of affording immunity against anthrax infection. Loewy and Richter[6] found that after producing a leucocytosis by means of injections of spermin, the resistance of the animal to Pneumococcus infection was increased. Jacob[7] found that animals in which he induced a hyperleucocytosis by subcutaneous injections of albumose showed marked resistance to infection with the Pneumococcus. R. Pfeiffer and A. Wassermann[8] showed that injections of serum rendered guinea-pigs immune to Cholera infection. Issaeff[9] reached similar conclusions. Wassermann[10] conferred resistance to infection by injections of physiological salt solution, urine, nuclein, etc.

Most important of all was the work of Hahn,[11] who found that dog's blood taken in the stage of hyperleucocytosis induced by subcutaneous injection of nuclein was more actively bactericidal than the normal blood from the same animal.

Their interpretations of these phenomena are not, however, in accord with one another, it being believed by some that the protective substances were simply brought to the point of inoculation by the leucocyte; by others that they are a product of leucocytic disintegration; by others that the protection is afforded through the reaction of other tissues, and makes itself manifest by the appearance in the blood of new properties that cannot as yet be referred with certainty to any specific type or group of cells as its origin.

Our own investigations * aimed to determine if the germicidal activity of the rabbit's serum as exhibited in vitro could be increased by the induction of an aseptic inflammatory reaction; if, like the hemolytic complement, the bacteriolytic complement is more evident in the circulating blood than elsewhere; and finally, if it can be reasonably referred to the leucocyte as its source.

In the course of the work there arose a number of other questions of interest, though of secondary importance. These will be discussed in their appropriate places.

The work was begun by a comparative study of the sera from normal and from treated rabbits from the standpoint of

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* Begun in October, 1902.

their bacteriolytic activity toward several bacterial species, namely, B. typhosus, B. coli communis, B. dysenteriæ, and Staphylococcus pyogenes aureus.

The animals designated as "treated" were rabbits in which an aseptic pleuritis had been induced by the intrapleural injection of sterile aleuronat suspensions. The blood of such animals was collected, as a rule, forty-eight hours after the intrapleural injection, and the serum used after the blood had stood in the ice chest over night. The bleeding of all animals was uniformly from the great vessels of the neck, using the method and apparatus recommended by Latapie.

The bacteriolytic tests were made by adding to the serum (whole or modified) varying amounts of the different bacteria upon which its activity was to be determined, and by making agar plates from such mixtures after varying intervals of time, the influence of the serum upon the bacteria being manifested by the number of colonies developing upon such plates. The colonies on the plates were counted after they had remained over night in the incubator at 37° C. To insure uniformity, the cultures to be used were not over twenty-four hours old, grown on agar agar at 35° C. From these, suspensions in salt solution (0.8 per cent) were carefully made, and from such suspensions the amount to be mixed with the serum was taken. Notwithstanding reasonable care, we found it impossible to convey uniformly from such a suspension into the serum the same number of bacteria, or even approximately the same number. To meet this difficulty all tests were made in duplicate and the mean of the results was taken as a satisfactory indicator.

For the analysis of the bacteriolytic phenomenon the customary plan was employed; *i.e.*, tests were made from a serum, first as derived from the rabbit, second after heating to 55° C. for thirty minutes, and third, with such heated serum reactivated with varying amounts of normal serum. The untreated serum is thus assumed to contain all factors needful to bacteriolysis, the heated serum only the amboceptors, and the normal serum used to reactivate the heated serum being regarded as consisting of complement only; an assumption which is, of course, not strictly true — though, as we supposed, near enough to the truth to serve our purpose. This opinion required modification. It will be discussed later on. All tests were carefully controlled by substituting for the serum under consideration tubes of physiological sodium chloride solution in amounts corresponding to that of the serum used.

The plan is perhaps best illustrated by table II., which shows that upon the bacillus typhosus sodium chloride solution in the proportions used is without effect; that normal serum destroys a very large proportion of the bacteria in three hours, and all in seventeen hours; that normal serum when heated for thirty minutes to 55° C. loses this germicidal activity; and that by the addition to such heated serum of unheated normal serum, in amounts ranging from 0.05 cc. to 0.2 cc. the bacteriolytic activity of the normal serum is practically restored; *i.e.*, its amboceptors for the typhoid bacillus find in such small amounts of normal serum sufficient complement to satisfy their haptophores, and thereby accomplish the destruction of the bacteria. That is to say, they are "reactivated."

In the tabulation of our results we have omitted, for the sake of brevity, a statement of the number of bacteria originally introduced into each tube of the test. After a fair study of this point we decided that the number of bacteria introduced into a control tube of salt solution underwent but little change during the first three or four hours of an experiment, and that therefore in any given comparative test the number of colonies developing from the control tube of salt solution after, say, one or two hours, represented approximately the number originally introduced not only into that tube, but also into all the other tubes of that series, made at the same time and under similar conditions.

THE AFFECT OF AN ASEPTIC PLEURITIS UPON THE GERMICIDAL ACTIVITY OF THE SERUM.

By the foregoing method a series of comparative tests were made with sera from normal rabbits and those from rabbits after intrapleural injection of aleuronat. The results were concordant in demonstrating the serum from the treated animals to be uniformly more actively germicidal than that from the normal animals.

Of forty experiments performed under this head with different species of bacteria, the following serve as fair samples of the results obtained:

TABLE I.*

	Number of Colonies on the Plates made after		
	1 hour.	2 hours.	18 hours.
With the bacillus typhosus:			
1. Control 0.8 per cent NaCl solution	44,285	46,200	∞
2. Normal serum 0.5 cc.	23,295	9,765	1,031
3. Aleuronat serum	155	2	0
With B. coli communis:			
1. Control 0.8 per cent NaCl solution	31,950	30,806	28,314
2. Normal serum	20,321	18,874	∞
3. Aleuronat serum	5,458	3,723	165
With B. dysenteriæ:			
1. Control 0.8 per cent NaCl solution	20,098	19,707	22,354
2. Normal serum	20,725	18,528	332
3. Aleuronat serum	18,278	9,567	0
With Staphylococcus pyogenes aureus:			
1. Control 0.8 per cent NaCl solution	7,014	5,927	∞
2. Normal serum	6,920	3,248	2,328
3. Aleuronat serum	2,325	401	688

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* For the sake of brevity the serum of treated animals will be designated "Aleuronat serum."

From the foregoing we see:

(1.) That both the normal and the aleuronat sera vary in their destructive action toward the several species of bacteria used in the test; and

(2.) That the aleuronat sera are uniformly more active than the normal sera.

The conclusion to be drawn from such results is obvious, viz.: that one at least of the consequences of an aseptic inflammatory reaction in a great serous cavity is a demonstrable increase in the germicidal activity of the animal's blood for the four organisms employed in the tests.

Since the germicidal activity of the sera of normal rabbits is known to vary in different individuals, objection might be raised to the adoption of such a standard of comparison. While our studies demonstrate such variations, we are not disposed to take them seriously in the light of a disturbing factor, since the tests with the aleuronat serum have, as stated, shown them to be uniformly more active than even the most active normal sera encountered by us.

Is the increase in germicidal activity referable to an increase of bacteriolytic complement?

If the increase of bactericidal power exhibited by the "aleuronat serum" is due to an increase of bacteriolytic complement, then theoretically a bacteriolytic serum rendered inactive by heating should have its activity restored more readily by "aleuronat" serum than by normal serum; *i.e.*, by smaller quantities of the former than of the latter.

For the elucidation of this point, as has been previously explained, the small amounts of unheated serum used for reactivation were assumed to contain only complement, and the differences in quantity needed for reactivation were regarded as due to differences in the complement content of the several sera employed. This assumption, as we shall see, is not fully warranted by what seem to be the facts.

At the very beginning of our work there was encountered a source of error that we have been unable fully to eliminate. In attempting to reactivate heated serum by the addition of small quantities of normal serum, it seemed possible for the reappearance of bactericidal power in the heated serum after such addition to have been due, after all, not to reactivation as such, but simply to the direct effect of the normal serum that was added. To decide this point, a number of control experiments were performed with sterile physiological salt solution, to which normal serum in small and varying amounts was added, the proportions maintained being the same as in the reactivation tests. The results were confusing. Several times the destruction of bacteria in salt solution, to which scarcely more than traces of normal serum were added, was as great as was observed in an equal bulk of undiluted normal serum, and was often greater than was observed in the reactivated sera.

Thus, for instance, in table II. appears the statement of an experiment designed to reactivate a heated serum with varying amounts of normal serum, Bacillus typhosus having been the test object; while in table III. is given the result of an analogous experiment in which similar quantities of normal serum were added to salt solution instead of to heated serum.

TABLE II.

	Number of Colonies on the Plates at the End of				
	At once.	1 hour.	3 hours.	6 hours.	18 hours.
1. Control NaCl solution........	5,895	6,498	7,677	7,906	12,159
2. Normal serum..............	2,599	575	3	1	0
3. Heated serum..............	6,633	12,043	14,130	Plates lost	36,579
4. Heated serum + .05 cc. normal serum....................	5,135	4,754	1,512	10	1
5. Heated serum + .1 cc. normal serum....................	7,325	3,265	28	2	2
6. Heated serum + .15 cc. normal serum....................	5,689	3,005	8	1	0
7. Heated serum + .2 cc. normal serum....................	5,832	1,320	9	1	0

In the former the results warrant the opinion that reactivation has been accomplished and is possible with relatively small amounts (0.05 cc.) of normal serum, but from the latter it is equally obvious that to some extent, though not conspicuously, the amounts of normal serum added for reactivation have *per se* some germicidal activity. Had this been constant a suitable subtractive correction might readily have been made; but as it is variable, we have found it impossible to decide upon a corrective factor, and have, therefore, discarded it; so that throughout all our reactivation tests this must be borne in mind. For a time we hoped to eliminate this error through the use of " pure complement," or at least of serum from which its bacteriolytic amboceptors had been removed by the plan of saturation with bacteria at low temperatures. As every effort in this direction resulted in failure, it was abandoned.

TABLE III.

	Number of Colonies on Plates after				
	At once.	1 hour.	3 hours.	6 hours.	21 hours.
1. Control NaCl.	1,633	1,417	1,334	1,169	1,004
2. Normal serum	2,520	837	504	122	7
3. Heated serum	2,366	2,079	2,646	2,475	4,473
4. Heated serum + .05 cc. nor. ser.	1,953	1,328	1,453	1,995	1,964
5. NaCl solution + .05 cc. nor. ser.	2,097	1,254	847	494	74
6. NaCl solution + .025 cc. nor. ser.	1,886	1,250	1,260	942	636
7. NaCl solution + .0125 cc. normal serum	1,354	1,000	978	588	902
8. NaCl solution + .005 cc. nor. ser.	1,758	1,260	1,300	995	4,678

At one time we thought that the reduction of bacteria in the salt solution to which only traces of normal serum had been added might have been only apparent, being referable to the agglutination of the bacteria by the highly diluted serum, and not to their destruction; the result of such agglutination being obviously a smaller number of colonies on the plates made from the mixture than would have been the case had the individual bacteria been evenly distributed throughout the fluid and not gathered together in clumps. We were unable, however, to secure evidence in support of this view, by either microscopic examination or naked eye observation of the original mixture in the test tube. That it is not a starvation phenomenon is obvious, since the reduction was always greater than in the control tests with salt solution alone.

Experiments made with fixed quantities (0.5 cc.) salt solution and amounts of normal serum ranging from 0.005 cc.–0.05 cc. did not give results to warrant the conclusion that the phenomenon is due to physical conditions. And a corresponding series with salt solution and heated instead of normal serum revealed the interesting fact that all germicidal activity disappeared completely. But if to sodium chloride solution was added a mixture of normal and of heated serum in the proportion of 0.0025 each of the latter to 0.5 cc. of the former, then more or less bacteriolysis is again observed. See table IV.

TABLE IV.

	Number of Colonies on Plates at End of				
	At once.	1 hour.	3 hours.	6 hours.	21 hours.
1. Control NaCl solution	3,307	3,395	3,359	3,370	3,420
2. Normal serum.................	2,488	130	2	0	0
3. Heated serum	2,425	3,399	3,463	....	3,759
4. Heated serum + .05 nor. ser...	3,150	2,192	1,840	409	240
5. NaCl solution + .05 cc. nor. ser.	3,050	2,350	2,753	1,055	1,156
6. NaCl solution + .025 cc. nor. ser.	2,992	2,238	2,233	2,047	∞
7. NaCl solution + .05 cc. normal heated serum	2,428	2,145	3,087	3,402	∞
8. NaCl solution + .025 cc. normal heated serum	2,521	2,170	2,842	3,385	∞
9. NaCl + .05 heated serum + .05 non-heated serum	2,452	1,921	455	207	15
10. NaCl + .025 heated serum + .025 non-heated serum	2,519	2,047	816	378	289

From our experiments we are forced to conclude that the small amount of serum used in reactivation is of itself capable of a certain variable degree of bactericidal activity. A true reactivation may be considered to take place in those cases in which the reactivated serum is more active than is a similar dilution of the reactivating serum in salt solution. Such activity in the reactivated serum cannot be uniformly obtained, probably because the complement and the intermediary body are not present in the correct proportions. The observations of Neisser and Wechsberg[12] indicate that when the intermediary body is largely in excess, bacteriolysis is partially prevented. It has been stated that this phenomenon can only be observed when immune serum is used, but our experiments indicate that at times, at least, it may be obtained with normal serum. For instance, in table V. are given results obtained upon Bacillus typhosus with a mixture containing a fixed amount (0.1 cc.) of normal serum and varying amounts (from 0.01 cc. to 1.0 cc.) of heated normal serum. Bacteriolytic activity is scarcely, if at all,

TABLE V.

	Heated Serum.	Normal Serum.	NaCl Sol.	Count of Plates made 3 hrs. after Inoculation.
1..........	1.0 cc.	0.1 cc.	0.9 cc.	∞
2..........	0.8 cc.	0.1 cc.	1.1 cc.	∞
3..........	0.6 cc.	0.1 cc.	1.3 cc.	40,000
4..........	0.4 cc.	0.1 cc.	1.5 cc.	1,900
5..........	0.2 cc.	0.1 cc.	1.7 cc.	1,200
6..........	0.1 cc.	0.1 cc.	1.8 cc.	45,000
7..........	0.01 cc.	0.1 cc.	1.89 cc.	∞
		Controls.		
8..........	0 cc.	0 cc.	2.0 cc.	∞
9..........	0.4 cc.	0 cc.	1.6 cc.	∞
10..........	0 cc.	1.0 cc.	1.0 cc.	35
11..........	0 cc.	0.1 cc.	1.9 cc.	50,000

appreciable in the tubes containing the higher and the lower quantities of heated serum; whereas, by comparison with the controls it is seen to be moderately active with mixtures containing amounts midway between the extremes. It is proper to state, however, that this result is not obtained with each trial. Many attempts made by us failed to demonstrate such relations.

Bearing these sources of error in mind, we may proceed to the consideration of table VI. which shows the results obtained in reactivation experiments performed with the object of determining whether the complement for the typhoid bacillus was increased in the serum of the "treated" animal.

TABLE VI.

	Number of Colonies on Plates after		
	1 hour.	2 hours.	18 hours.*
With B. typhosus:			
1. Control NaCl solution	45,170	47,400	∞
2. Control NaCl + .1 cc. normal serum	37,000	30,000	1,890
3. Control NaCl + .1 cc. aleuronat serum	7,875	540	0
4. Control normal serum	22,650	11,340	1,110
5. Heated normal serum + .1 cc. normal serum	75,000	60,000	80,000
6. Heated aleur. serum + .1 cc. normal serum	70,000	50,000	65,000
7. Heated normal serum + .1 cc. aleur. serum	17,010	5,670	0
8. Heated aleur. serum + .1 cc. aleuronat serum	16,217	3,780	40

*The entire tube was used for this plate. For the plates made at the end of one and two hours, two oese were taken from the salt solution or serum mixture.

In the foregoing table VI. we have in tube five half a cubic centimeter of heated normal serum, and in tube six half a cubic centimeter of heated aleuronat serum. To each of these sera 0.1 cc. of normal serum was added for the purpose of reactivation.

Now if the complement is the factor that is increased in the aleuronat serum, it should be entirely destroyed by the heating, and there should be no difference in the bactericidal action between the two tubes five and six, which is practically the case, both being almost nil. If, on the other hand, it is the intermediary body that is increased, heating the serum would have no effect upon this substance and we should expect a greater bactericidal action in the tube six containing the heated aleuronat serum, than in tube five containing the heated normal serum. But in the experiment the germicidal action of tubes five and six was about the same, and certainly not marked, and this would indicate that it is the complement that is increased in the aleuronat serum. Let the plan of analysis now be reversed: tube seven contains heated normal serum, and tube eight contains heated aleuronat serum; both are reactivated with .1 cc. aleuronat serum. If it is the complement that has been increased in the treated animals' serum, we should expect that both tubes seven and eight would exhibit greater bactericidal action than the tubes five and six; this we show to be the case. But if it is the intermediary body that is increased, we may expect that bacteriolysis will be greater in tube eight than in tube seven. In the experiment it will be seen that the bacteriolytic action of tubes seven and eight is about alike, and that it is much greater than in tubes five and six. We conclude, therefore, that the increased germicidal activity of the aleuronat serum may reasonably be regarded as due to increase of bacteriolytic complement.

Table VII. gives similar experiments with the other organisms.

From this exhibit (table VII.) it is plain that efforts at reactivation are much less successful when B. coli communis, B. dysenteriæ, and Staphylococcus pyogenes aureus are the test objects, than when the behavior of the serum is observed upon Bacillus typhosus. With B. dysenteriæ the effort was to some extent successful, but with the other species the figures given fairly represent our failures.

TABLE VII.

	Number of Colonies on Plates after		
	2 hours.	4 hours.	24 hours.
With the B. coli communis:			
1. Control NaCl solution	14,327	18,760	20,195
2. Control NaCl + .1 cc. normal serum	17,400	15,321	95,000
3. Control NaCl + .1 cc. aleur. serum	7,825	3.240	24,185
4. Control normal serum	10,770	6,870	35,164
5. Heated nor. serum + .1 cc. nor. serum	14,215	15,140	91,000
6. Heated aleur. serum + .1 cc. nor. serum	15,119	16,920	37,800
7. Heated nor. serum + .1 cc. aleur. serum	13,000	11,531	96,000
8. Heated aleur. serum + .1 cc. aleur. serum	12,765	7,560	19,200
With the B. dysenteriæ:			
1. Control NaCl solution	10,675	14.739	11,838
2. Control NaCl + .1 cc. normal serum	10,263	14,580	∞
3. Control NaCl sol. + .1 cc. aleur. serum	11,131	5,400	498
4. Control normal serum	12,960	17,205	212
5. Heated nor. serum + .1 cc. nor. serum	11,130	9,180	1,150
6. Heated aleur. serum + .1 cc. nor. serum	12,340	12,000	932
7. Heated nor. serum + .1 cc. aleur. serum	938	8,020	160
8. Heated aleur. serum + .1 cc. aleur. serum	10,739	2,700	18
With Staphylococcus pyogenes aureus:			
1. Control NaCl solution	8,245	4,960	∞
2. Control NaCl + .1 cc. normal serum	6,400	7,620	∞
3. Control NaCl + .1 cc. aleur. serum	4,120	7,315	100,000
4. Control normal serum	6,740	2,520	3,230
5. Heated normal serum + .1 cc. normal serum	4.340	4,180	14.450
6. Heated aleuronat serum + .1 cc. normal serum	4,650	3,160	6,510
7. Heated normal serum + .1 cc. aleuronat serum	3,300	2,790	6,718
8. Heated aleuronat serum + .1 cc. aleur. serum	2,520	756	3,119

For obvious reasons only a few experiments can be given in detail, but the following is a summary of the results: Of eleven treated animals whose sera was tested with B. typhosus, eleven showed increased bactericidal action. The serum of seven treated animals was tested with B. coli communis, and all showed increased bactericidal action. The serum from seven treated animals was tested with B. dysenteriæ, and seven showed increased germicidal activity. The serum of thirteen treated animals was tested with Staphylococcus pyogenes aureus, of which twelve showed an increase in bactericidal activity, and one failed to show such an increase.

Of eight treated animals tested for an increase of complement for the typhoid bacillus, eight showed such an increase. Of seven animals similarly tested for B. coli communis, seven showed an increase in bacteriolytic complement. Of seven tested for B. dysenteriæ, seven showed an increase of complement for that bacillus; and of thirteen treated animals tested with Staphylococcus pyogenes aureus, eleven showed an increase of complement content, while two showed no increase.

Of eight treated animals tested for increase of intermediary body for B. typhosus, three showed an increase, while five showed none. Of seven sera from treated animals so tested for B. coli communis, six showed an increase, while one did not. Seven treated animals were tested with B. dysenteriæ. Three showed an increase of intermediary body, while four showed no such increase. Thirteen sera from treated animals were tested for increase of intermediary body for Staphylococcus pyogenes aureus. Eleven of them demonstrated such an increase, while two did not.

IS THE COMPLEMENT DERIVED FROM THE LEUCOCYTE?

An increase in complement content, then, is brought about in the serum by setting up in the animal a reaction whose most conspicuous feature is a considerable local leucocytosis. This fact suggested the possible leucocytic origin of the complement.

The literature on this subject is too extensive and too well-known to be given here. Much of it antedates the analytical methods in use at present. Quite recently L. Ascher[13] in a study of the relation of the leucocyte to the complement for the cholera organism finds that the complement is present in the circulation, and is not derived from the leucocytes. Among other experiments upon which he bases this view, Ascher injects aleuronat into the pleural cavity, and following Buchner's method obtains an extract of the leucocytes from the exudate so produced. He finds that the complement is all present in the centrifugated exudate fluid, and that there is no complement whatever in the leucocyte extract. He concludes, "That there is no ground for supposing that the leucocytes have the slightest relation to the sources of the complement for Cholera."

In order to obtain evidence upon this point three methods were pursued by us:

(1.) The comparison of the complement content of the leucocytic extract with that of the serum of the same animal.

(2.) The comparison of the complement content of the serum with that of the aqueous humor.

(3.) The comparison of the complement content of the serum with that of the so-called plasma.

(1.) Comparison of the complement content of the leucocytic extract with that of the serum of the same animal.

The leucocyte extract was obtained according to the method of Buchner from the washed leucocytes obtained by centrifugation of a pleural exudate withdrawn forty-eight hours after the injection of aleuronat suspension. At this time the polymorphonuclear leucocytes are the predominating element in such an exudate. The fluid portion of the exudate was likewise examined, and its action compared with that of the serum of the same animal. An example of such an experiment with each of the organisms studied is shown in table VIII.

TABLE VIII.

	Number of Colonies on Plates after		
	2 hours.	4 hours.	24 hours.
With Staphylococcus pyogenes aureus :			
1. Control NaCl solution	4,872	5,242	∞
2. Serum	231	122	90
3. Exudate fluid	4,320	1,080	1,512
4. Heated serum + .1 cc. exudate fluid	632	116	142
5. Leucocyte extract	4,216	3,780	30,500
6. Heated serum + .1 cc. leucocyte extract	986	126	212
With B. typhosus :			
1. Control NaCl solution	50,000	55,000	∞
2. Serum	92	2	0
3. Exudate fluid	912	48	0
4. Heated serum + .1 cc. exudate fluid	42,000	18,900	22,640
5. Heated exudate fluid + .1 cc. serum	13,500	34	0
6. Leucocytic extract	2,400	230	725
7. Heated serum + .1 cc. leucocyte extract	38,000	40,000	∞
8. Heated leucocyte extract + .1 cc. serum	242	4	5
With B. dysenteriæ :			
1. Control NaCl solution	9,265	10,125	8,230
2. Serum	9,890	2,415	4
3. Exudate fluid	10,868	10,580	40,000
4. Heated serum + .1 cc. exudate fluid	9,785	9,460	8,342
5. Heated exudate fluid + .1 cc. serum	10,636	10,892	42,000
6. Leucocyte extract	9,549	10,315	50,000
7. Heated serum + .1 cc. leucocyte extract	10,730	10,125	∞
8. Heated leucocyte extract + .1 cc. serum	9,382	10,431	∞
With B. coli communis :			
1. Control NaCl solution	10,842	9,463	10,897
2. Serum	934	867	82

TABLE VIII. — *Continued.*

	Number of Colonies on Plates after		
	hours.	4 hours.	24 hours.
3. Exudate fluid	10,182	8,683	8,129
4. Heated serum + .1 cc. exudate fluid	6,480	6,124	40,000
5. Heated exudate fluid + .1 cc. serum	13,540	7,189	6,195
6. Leucocyte extract	2,173	8,285	∞
7. Heated serum + .1 cc. leucocyte extract	4,829	5,468	∞
8. Heated leucocyte extract + .1 cc. serum	2,464	2,385	∞

From these experiments we may conclude:

(*a.*) That the complement for Staphylococcus pyogenes aureus is found both in the exudate fluid and in a leucocytic extract, and therefore may be, in part at least, derived from the leucocytes. There is no evidence that there is more complement in the leucocytic extract than in the serum.

(*b.*) That the complement for B. typhosus is not contained in the leucocyte extract of the exudate fluid, while both of these fluids contain intermediary body for the typhoid bacillus.

(*c.*) That neither complement nor intermediary body for B. dysenteriæ are present in either the exudate fluid or the leucocytic extract.

(*d.*) That neither complement nor intermediary body for B. coli communis are present in the exudate fluid or in the leucocytic extract.

(2.) Comparison of the amount of complement in the serum and the aqueous humor.

Experiments to determine the presence of the bacteriolytic complement in the aqueous humor were next undertaken. A treated animal was anesthetized, and the aqueous humor withdrawn with a sterile syringe. In certain cases this fluid formed a very slight coagulum. After the anterior chambers of the eyes had again filled, the newly-formed aqueous humor

TABLE IX.

	Number of Colonies on Plates after		
	2 hours.	4 hours.	24 hours.
With the Staphyloccocus pyogenes aureus:			
1. Control NaCl solution	3,611	4,464	∞
2. Serum	1,522	216	231
3. Aqueous humor	1,892	3,110	18,200
4. Heated serum + .1 cc. aqueous humor	2,118	792	2,080
5. New aqueous humor	3,576	3,240	21,600
6. Heated serum + .1 cc. new aqueous humor	1,746	640	6,480
With B. typhosus:			
1. Control NaCl solution	47,200	52,000	∞
2. Serum	120	3	0
3. Aqueous humor	50,000	47,000	∞
4. Heated serum + .1 cc. aqueous humor	45,000	42,000	∞
5. New aqueous humor	21,600	15,400	8,250
6. Heated serum + .1 cc. new aqueous humor	35,000	25,200	∞
With B. dysenteriæ:			
1. Control NaCl solution	9,265	10,125	8,230
2. Serum	5,870	2,150	8
3. Aqueous humor	10,122	10,875	9,120
4. Heated serum + .1 cc. aqueous humor	10,351	10,465	∞
5. New aqueous humor	9,965	6,490	∞
6. Heated serum + .1 cc. new aqueous humor	7,340	9,320	15,000
With B. coli communis:			
1. Control NaCl solution	10,643	8,973	7,886
2. Serum	1,432	1,146	106
3. Aqueous humor	8,692	8,854	∞
4. Heated serum + .1 cc. aqueous humor	5,574	7,434	∞
5. New aqueous humor	7,785	8,763	∞
6. Heated serum + .1 cc. new aqueous humor	7,548	9,421	∞

was withdrawn. This fluid was diluted with an equal bulk of physiological sodium chloride solution, after which coagulation took place. The serum obtained from this coagulum, the normal aqueous humor, and the serum were tested as to their comparative complement content. Experiments with each of the organisms are detailed in table IX.

From these experiments we may conclude:

(*a.*) That the complement for Staphylococcus pyogenes aureus is present, to some extent, in both the normal aqueous humor and in the aqueous humor of new formation, though in smaller quantities than in the serum of the same animal.

(*b.*) That the complements for B. typhosus, B. coli communis, and B. dysenteriæ are not present in either normal or newly formed aqueous humor.

(*c.*) That the bacteriolytic complements differ from one another in their ability to pass through an animal membrane.

(3.) Comparison of the amount of complement in the serum and in the plasma.

The so-called plasma was obtained by the method used by Bordet and Gengou.[14] The bactericidal action of these fluids was compared as shown in table X.

TABLE X.

	Number of Colonies on Plates after		
	2 hours.	4 hours.	24 hours.
With B. typhosus:			
1. Control NaCl solution	10,865	12,483	21,640
2. Serum	1	0	0
3. Plasma	4	3	0
4. Heated serum + .1 cc. plasma	1,035	352	2
5. Heated serum + .1 cc. serum	2,160	361	3
With B. coli communis:			
1. Control NaCl solution	4,520	8,240	20,520
2. Serum	660	2	0
3. Plasma	434	0	0

It seems as the result of these experiments that the bactericidal action and the complement content of the serum and the plasma are the same.

We realize full well that the body designated as plasma is not plasma in the strict sense, but we believe it is as near plasma as it is possible to get in a test tube. With reasonable care blood collected by the method here used may be quickly rid of all cellular elements before even beginning coagulation, so that the coagulation that may subsequently occur if dependent upon or associated with the disintegration of leucocytes must involve them in very small numbers.

ON THE MULTIPLICITY OF THE BACTERIOLYTIC COMPLEMENT.

In this series of experiments an attempt was made to obtain some evidence with regard to the multiplicity of the bacteriolytic complement. If these complements are multiple, it should be possible to separate them as has already been done for some of the hemolytic complements by Ehrlich and Morgenroth [15] and Neisser and Doering.[16]

Filtration of the serum with the Chamberland and Berkefeld filters was the method adopted, in the hope that the complements for some bacteria might pass through, while those for others might be retained upon the filter. An experiment in which the bactericidal action of the filtrate from the Chamberland filter was compared with that of the normal serum is shown in table XI.

TABLE XI.

	Number of Colonies on Plates after		
	1 hour.	3 hours.	24 hours.
With B. typhosus:			
1. NaCl solution control	21,172	20,740	18,752
2. Normal serum	14,400	6,321	38
3. Filtered serum	20,325	16,211	∞

TABLE XI. — *Continued.*

	Number of Colonies on Plates after		
	1 hour.	3 hours.	24 hours.
4. Filtered serum + .1 cc. normal serum	10,800	4,125	1,241
5. NaCl solution + .1 cc. normal serum	15,128	13,550	19,324
With B. dysenteriæ :			
1. Control NaCl solution	2,718	1,820	3,127
2. Normal serum	2,523	206	0
3. Filtered serum	5,276	8,765	∞
4. Filtered serum + .1 cc. normal serum	2,473	150	721
5. NaCl solution + .1 cc. normal serum	2,792	480	660
With Staphylococcus pyogenes aureus :			
1. Control NaCl solution	4,440	5,828	1,540
2. Normal serum	4,129	141	6
3. Filtered serum	3,976	5,473	∞
4. Filtered serum + .1 cc. normal serum	6,820	5,940	984
5. NaCl solution + .1 cc. normal serum	6,427	5,311	6,132
With B. coli communis :			
1. Control NaCl solution	2,174	1,894	4,321
2. Normal serum	840	632	34
3. Filtered serum	6,480	10,746	∞
4. Filtered serum + .1 cc. normal serum	721	612	∞
5. NaCl solution + .1 cc. normal serum	1,040	918	28,000

It will be seen from this table that the filtered serum has lost its germicidal power for all four organisms. This was the result uniformly obtained with the Chamberland filter. But the results obtained by comparing the filtrate from the Berkefeld filter with the normal serum were different, and are given in table XII.

By passage through this filter, the serum has been entirely deprived of its bacteriolytic action upon B. coli communis and Staphylococcus pyogenes aureus, and partly deprived of its action for B. dysenteriæ. On the other hand, the bactericidal power of the filtered serum for B. typhosus remains unimpaired. It is possible to reactivate this filtered serum, and therefore this loss of germicidal activity for three of the four organisms must be due to a loss of bacteriolytic complement.

TABLE XII.

	Number of Colonies on Plates after		
	2 hours.	4 hours.	24 hours.
With B. typhosus:			
1. Control with NaCl solution	18,912	19,135	20,325
2. Normal serum	315	1	0
3. Filtered serum	2,716	3	0
4. Filtered serum + .1 cc. normal serum	116	0	0
5. NaCl solution + .1 cc. normal serum	1,252	14	1
With B. coli communis:			
1. Control NaCl solution	2,785	2,895	2,681
2. Normal serum	1,931	916	1,241
3. Filtered serum	2,564	1,533	∞
4. Filtered serum + .1 cc. normal serum	896	710	10,800
5. NaCl solution + .1 cc. normal serum	1,020	971	16,200
With B. dysenteriæ:			
1. Control NaCl solution	2,621	4,879	6,591
2. Normal serum	780	362	1
3. Filtered serum	8,215	8,455	1,624
4. Filtered serum + .1 cc. normal serum	1,380	1,810	0
5. NaCl solution + .1 cc. normal serum	1,873	5,324	2,475
With Staphylococcus pyogenes aureus:			
1. Control NaCl solution	2,083	2,961	5,431
2. Normal serum	1,542	73	675
3. Filtered serum	1,831	4,780	∞
4. Filtered serum + .1 cc. normal serum	1,680	1,835	20,100
5. NaCl solution + .1 cc. normal serum	2,342	1,473	2,160

From these observations and those of others noted above, it seems possible to separate the bacteriolytic complements from one another by the process of filtration.

Separation of the agglutinins. — A series of experiments upon the germicidal properties of some immune sera obtained from rabbits immunized to B. typhosus was performed. As a result of these experiments it may be stated that the bactericidal action of these immune sera was no greater than that of the normal serum.

This immune serum was also filtered through the Chamberland filter. The filtrate thus obtained was tested as shown in the following table XIII.:

TABLE XIII.

	Number of Colonies on Plates after		
	2 hours.	4 hours.	24 hours.
With B. typhosus:			
1. Control NaCl solution	13,500	15,362	16,124
2. Immune serum	26	0	0
			Agglutination
3. Filtered immune serum	16,215	17,231	28,136
	Controls with B. coli communis		
4. NaCl solution	10,324	12,953	8,720
5. Immune serum	2,731	1,652	40,300
6. Filtered immune serum	11,189	13,425	∞

It had entirely lost its bacteriolytic action, but it was observed that it produced a marked agglutination, evident both to the naked eye and by microscopic examination. There was no clumping to be observed, either with the naked eye, or with the microscope in the tubes of unfiltered immune serum, or in control tubes inoculated with the colon bacillus.

The explanation of this phenomenon is probably that the agglutinins produced in the immunization of the animal pass through the Chamberland filter, while the bacteriolytic complements are removed by filtration as has been shown. In the unfiltered immune serum, agglutination is not observed because bacteriolysis occurs too rapidly.

We may conclude, therefore, that the bacteriolytic substances and the agglutinins may be separated by filtration.

ARE THE HEMOLYTIC AND THE BACTERIOLYTIC COMPLEMENTS SEPARATE?

If the hemolytic and bacteriolytic complements are identical, the bactericidal action of the serum should disappear when the hemolytic complement is exhausted.

Rabbits' serum was, therefore, treated with washed guinea-pigs' erythrocytes, until hemolysis no longer occurred, and

TABLE XIV.

	Number of Colonies on Plates after		
	1 hour.	3 hours.	24 hours.
With B. typhosus:			
1. Control NaCl solution	47,200	52,000	∞
2. Normal serum	120	3	1
3. Serum after hemolysis	132	11	3
With Staphylococcus pyogenes aureus:			
1. Control NaCl solution	4,872	5,242	∞
2. Normal serum	240	176	95
3. Serum after hemolysis	4,540	9,482	∞
With B. coli communis:			
1. Control NaCl solution	6,866	8,341	10,324
2. Normal serum	2,421	5,450	240
3. Serum after hemolysis	7,325	6,744	34,317
With B. dysenteriæ:			
1. Control NaCl Solution	9,265	10,125	8,230
2. Normal serum	9,890	2,415	4
3. Serum after hemolysis	9,118	6,128	38,000

thus the hemolytic complement for those corpuscles was presumably exhausted. The bacteriolytic action of this fluid was then tested. See table XIV.

It was found that this fluid was as actively bacteriolytic for typhoid bacilli as was the normal serum, but that when it was tested upon B. coli communis, B. dysenteriæ, or Staphylococcus pyogenes aureus, no evidences of bacteriolysis were obtained. On the contrary, these three species grew luxuriantly in such serum. But further experiments led us to regard this loss of bacteriolytic power as apparent rather than real, and to suspect that bacteriolysis was only masked by the growth of the surviving organisms. This rapid growth was probably stimulated by the presence of nutritive substances derived from the dissolved red corpuscles — a phenomenon previously observed by Buchner.[17] We have little doubt that this is the correct explanation of the apparent loss of bactericidal power for B. coli communis, B. dysenteriæ, and Staphylococcus pyogenes aureus in the serum in which hemolysis had previously occurred; for the same apparent loss of germicidal action may be observed, if instead of the guinea-pig corpuscles, a solution of these corpuscles, or better still, of rabbits' corpuscles in distilled water, be added to the rabbits' serum. Whether such a solution can have an effect upon either hemolytic or bacteriolytic complements cannot be said; but the addition of it to a germicidal serum induces sufficiently active growth of bacteria in that serum as to mask entirely such destructive or restraining power as it may possess.

Further evidence that the hemolytic and bacteriolytic complements are distinct is gained from experiments in which the amount of bacteriolytic complement in rabbit's serum was diminished by adding to the serum bacteria for which it is destructive, and then testing such serum upon guinea-pigs' erythrocytes for which it is normally hemolytic. No loss of hemolytic activity could be subsequently demonstrated in sera tested by us in this manner. These results support, therefore, the opinion that the hemolytic and bacteriolytic complements are not identical.

CONCLUSIONS.

As a result of the experiments just described, we are led to conclude:

1. That the production of an aseptic inflammatory reaction is followed by a demonstrable increase in the bactericidal activity of the animal's serum.

2. That this increased germicidal activity is due to an increase in the bacteriolytic complements in amounts that vary more or less according to the organism and the particular serum tested. In certain sera an increase in the amount of amboceptor can also be demonstrated.

3. That although in the case of Staphylococcus pyogenes aureus a certain amount of the complement seems to be referable to the leucocyte, there seems to be no evidence in favor of the leucocytic origin of the complements for the other organisms tested.

4. That in their relations to animal membranes the bacteriolytic complements differ from one another, some being able to pass through by dialysis, others not.

5. That the complements for all the organisms tested may be separated from the serum by filtration through a Chamberland filter; but by passage through a Berkefeld filter the complements for certain organisms are retained, while those for others pass through, a further indication of the multiplicity of the bacteriolytic complement.

6. That by filtration through a Chamberland filter it is possible to separate the agglutinins from the bactericidal substances of a serum.

7. Since the destruction of bacteria by a serum that is both bacteriolytic and hemolytic in no way interferes with its hemolytic properties; and since the destruction by that serum of erythrocytes does not interfere with bacteriolysis, it is highly probable that the hemolytic and bacteriolytic complements are separate and independent.

(In conclusion, I wish to express my great obligation to Dr. A. C. Abbott, the director of the laboratory, who proposed to me this subject for investigation, and whose suggestions and advice have rendered the work possible.)

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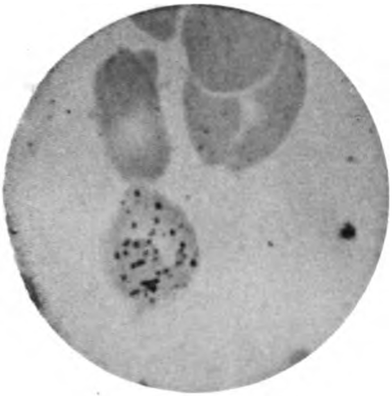
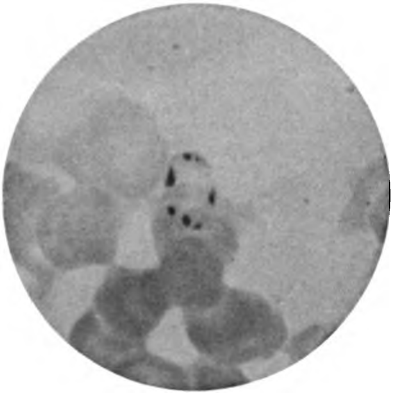


1

Blue stippling, coarse. Slight suggestion of rings. Pernicious Anemia. Prof. B.



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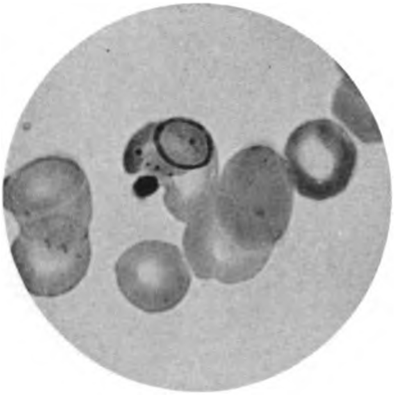
Lead Poisoning, No. 12611. Heavy blue masses at the periphery of a red cell.



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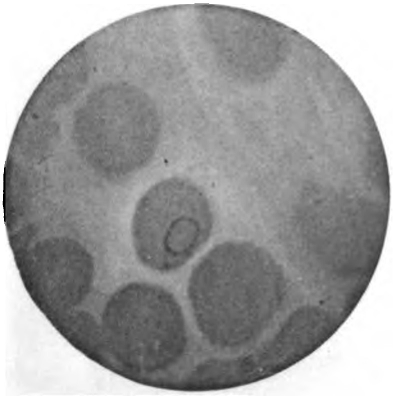
Lead Poisoning, No. 12611. Red ring.



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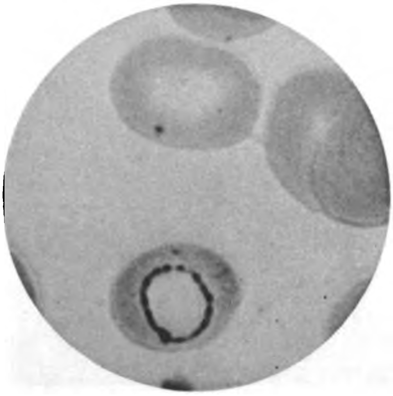
Mrs. B. Pernicious Anemia. Red ring. No stippling.



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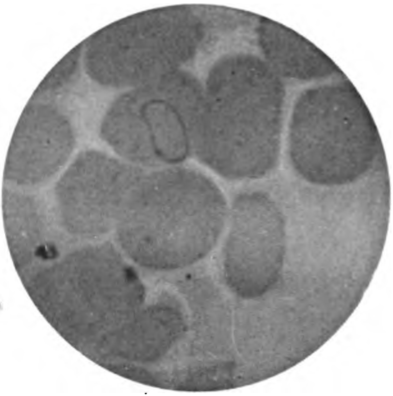
Lead Poioning. No. 12611. Red ring. No stippling.

R.C.Cabot.

Anemic Blood.

[illegible] Type Co., Boston.



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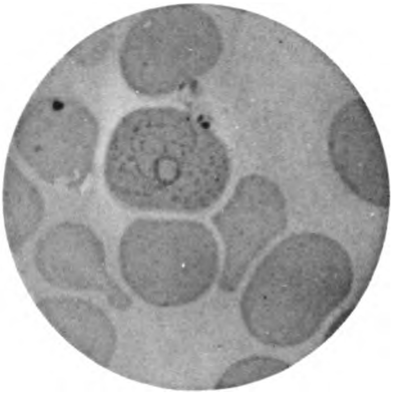
Red ring, no stippling, Pernicious Anemia.
Prof. B.



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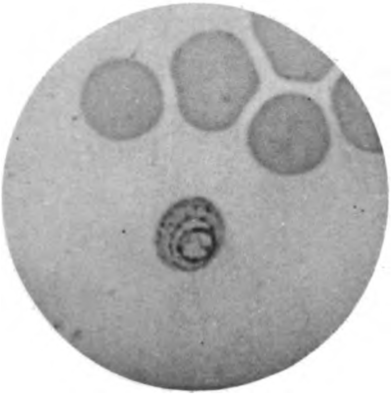
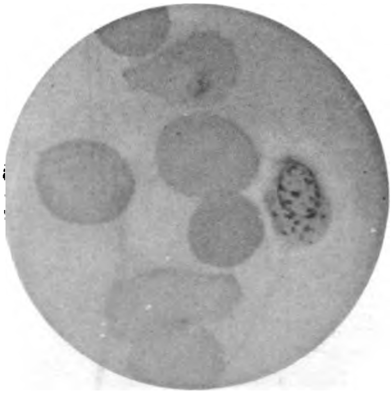
Small red ring with stippling, Pernicious
Anemia. Mrs. B.



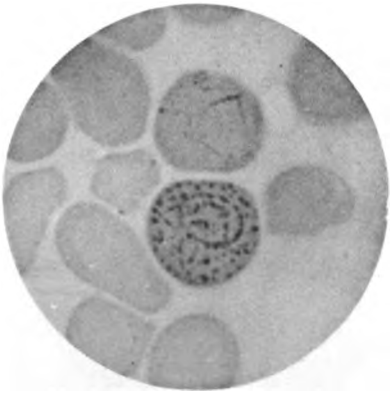
8

One red ring containing a second red stippling.
Pernicious Anemia. Mrs. B.



9

Blue ring and stippling, Corpuscle fainter staining
than its neighbors. Pernicious Anemia. Prof. B.

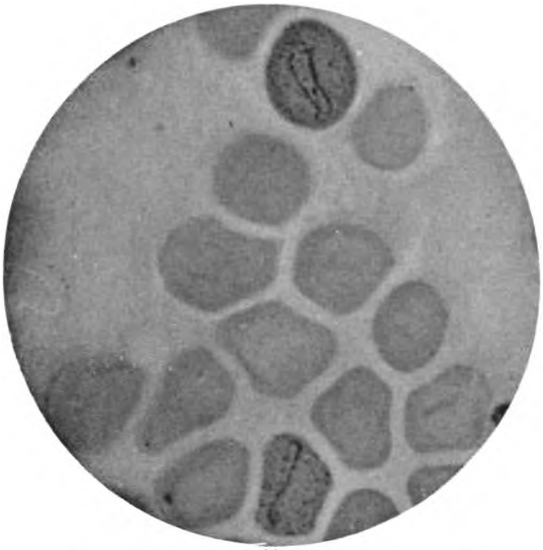


10

The upper cell contains a faint red ring. The
lower has blue stippling and a blue ring.

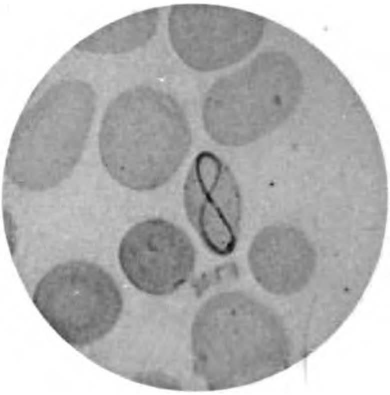
R. C. Cabot. Anemic Blood.

Heliotype Co., Boston.



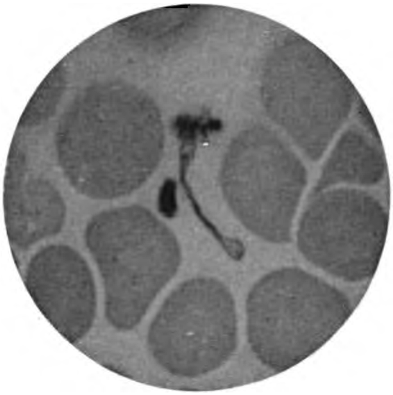
11

Mrs. B. Pernicious Anemia. Two cells containing red rings more or less twisted and red stippling.



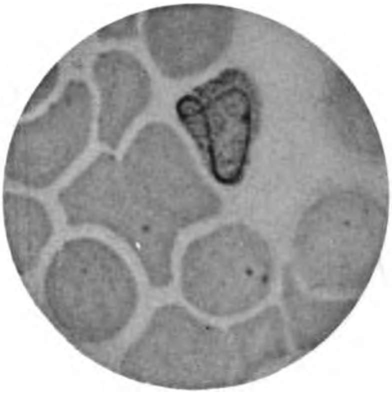
12

Mrs. B. Pernicious Anemia. Red ring, twisted. Traces of stippling.



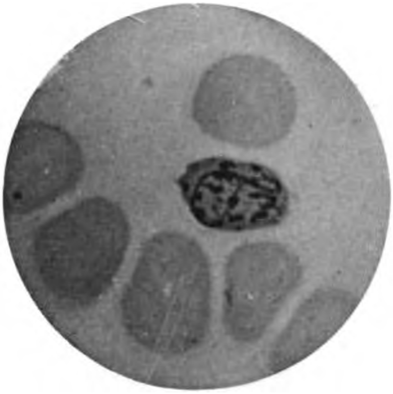
13

Red staining extracellular body apparently a twisted ring. Two blood-placques near.



14

Red ring. Blue stippling. Cell-body, pale blue. Pernicious Anemia Mrs. B.



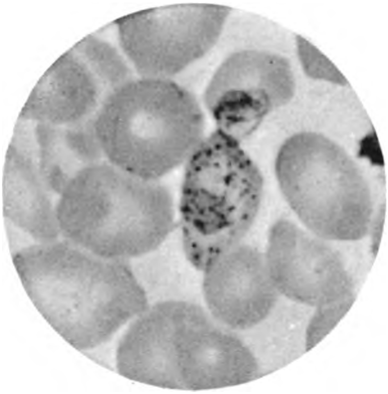
15

Figure of eight, cherry red. Other markings, dark blue. Cell-body, light pink. Mrs. B. Pernicious Anemia.

R C Cabot.

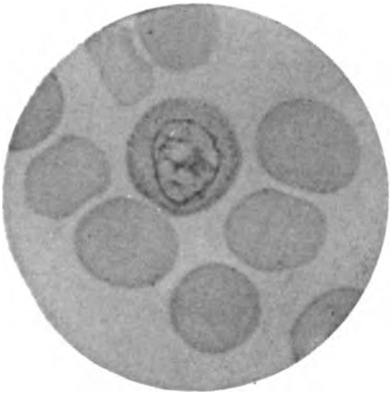
Anemic Blood

Heliotype Co., Boston



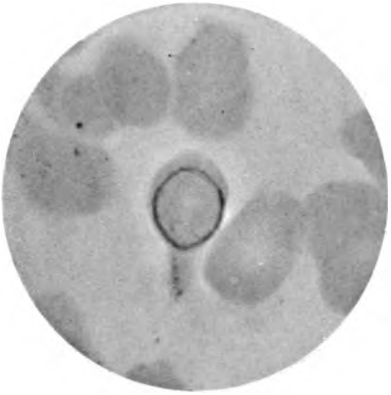
16

Prof. B. Pernicious Anemia. Red stippling and rings, partially extra cellular.



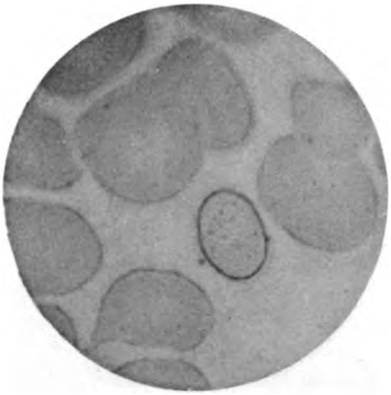
17

Red ring with net work within it. Red stippling, Mrs. B. Pernicious Anemia.



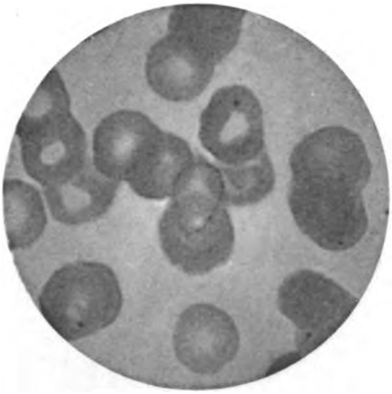
18

Red ring. No stippling.
Prof. B. Pernicious Anemia.



19

Red ring near periphery of cell. Somewhat similar appearance in neighboring cells. Pernicious Anemia.

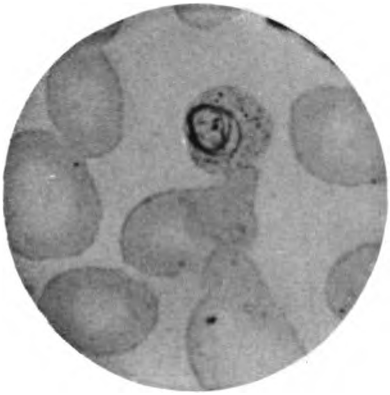


20

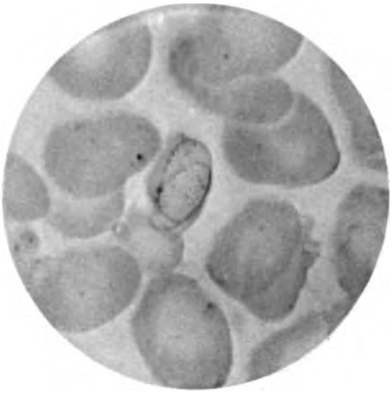
Lead Poisoning. No. 12611. Red ring. Apparently but not really extra cellular.

R.C.Cabot. Anemic Blood.

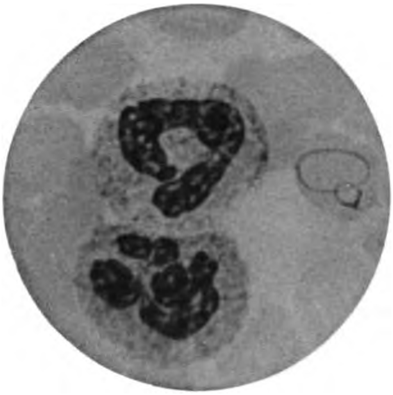
Heliotype Co., Boston.



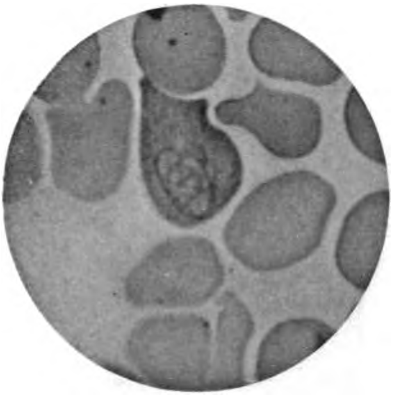
21
Multiple intertwined red rings with red stippling.
Pernicious Anemia. Prof. B.



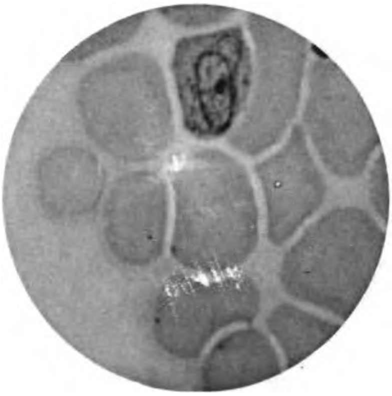
22
Two intersecting red rings.
Pernicious Anemia. Prof. B.



23
Mrs. B. Pernicious Anemia. Red rings.
No stippling. Two polynuclear leucocytes.



24
Red rings arranged like a "clover leaf" nucleus.
Red stippling. Prof. B. Pernicious Anemia.

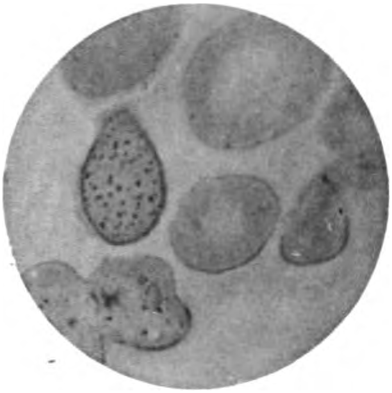


25
Interlacing red rings with one large dot.
Nuclear remnant? Pernicious Anemia. Prof. B.

R. C. Cabot.

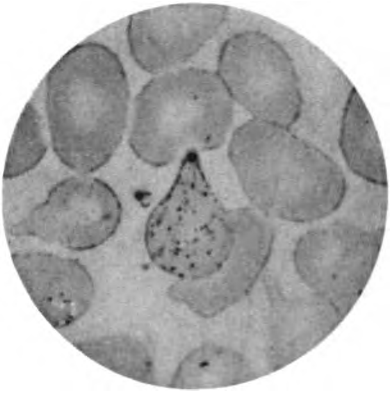
Anemic Blood.

Heliotype Co., Boston.



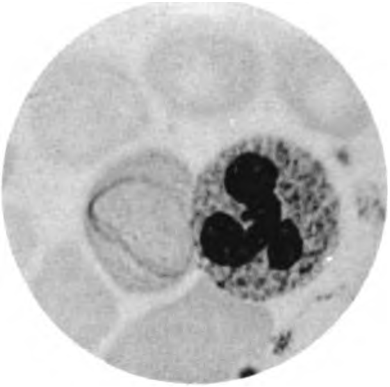
26

Red ring near outer margin of cell. On the left a trace of the cell body projects beyond the ring.



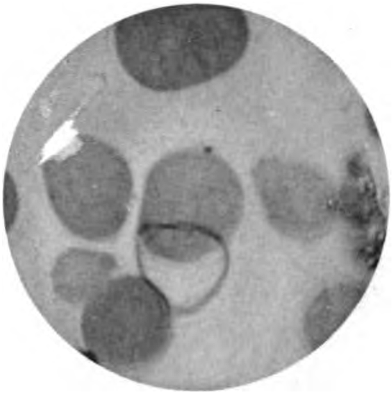
27

Red stippling and bordering with a single large red dot. Similar appearance in neighboring cells.



28

Red ring and faint stippling, twisted thread appearance in ring. Pernicious Anemia. Mrs. B.



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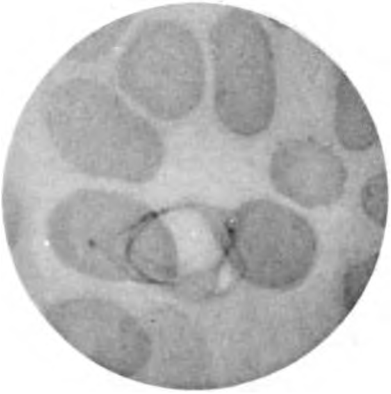
Red rings, extracellular.
Prof. B. Pernicious Anemia.

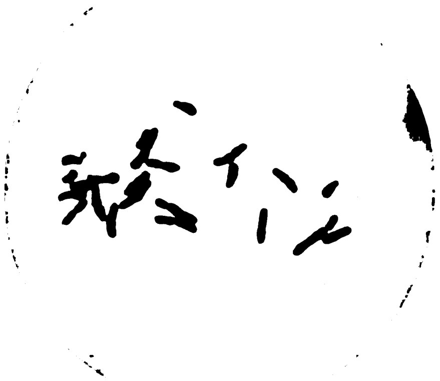


30

Four twisted red rings extracellular but apparently identical with the intracellular rings. Mrs. B.
Pernicious Anemia.

R. C. Cabot. Anemic Blood

Heliotype Co., Boston.



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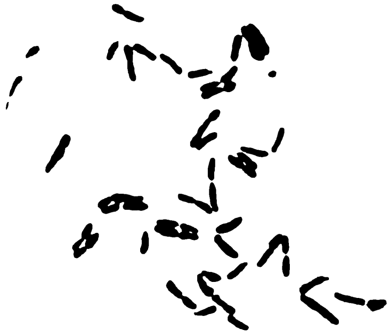

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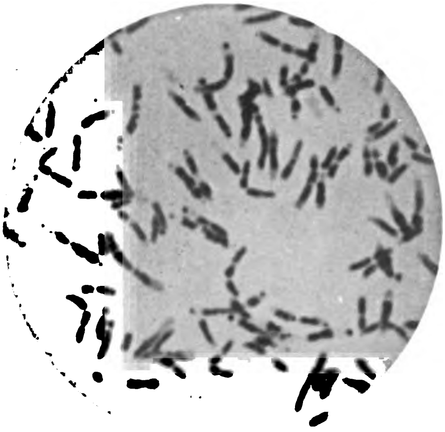

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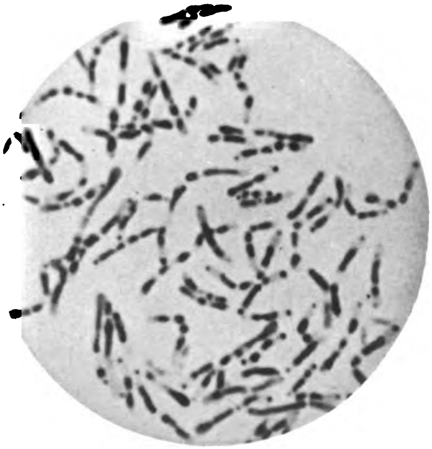

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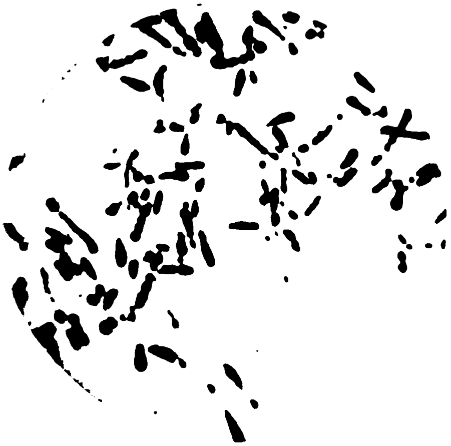

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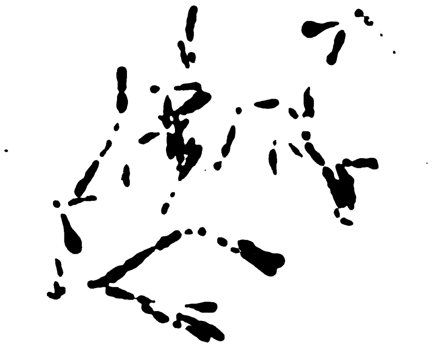
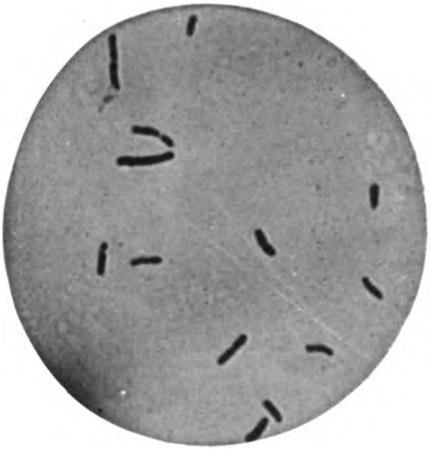


6

Denny.

B. Diphtheriae, etc.



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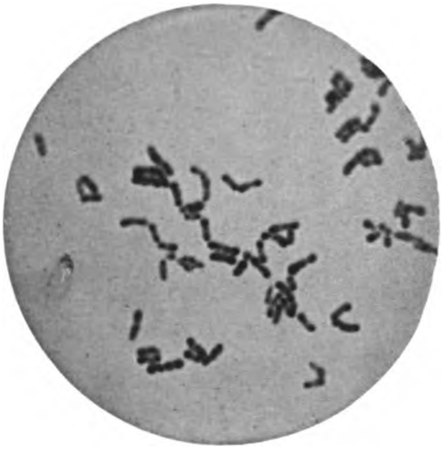

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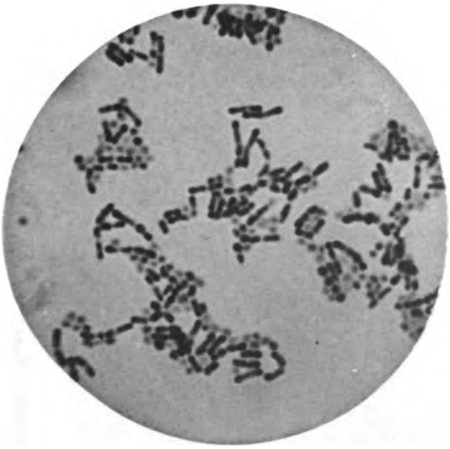

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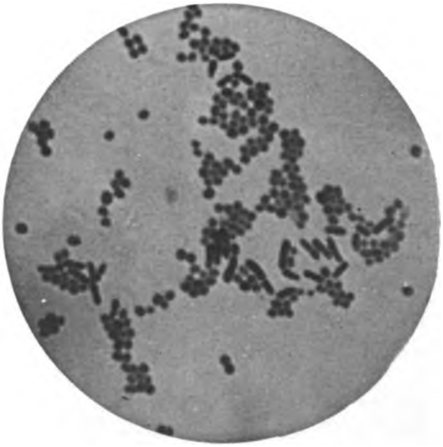

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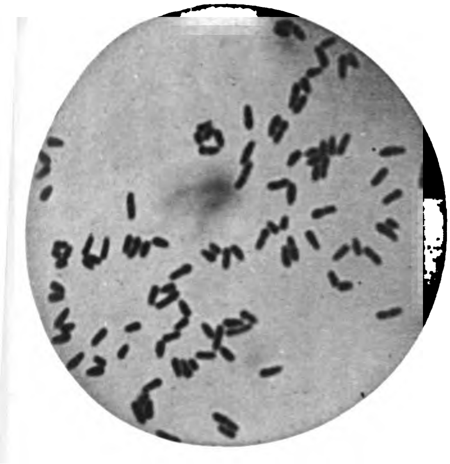
B. Diphtheriae, etc.

Denny.



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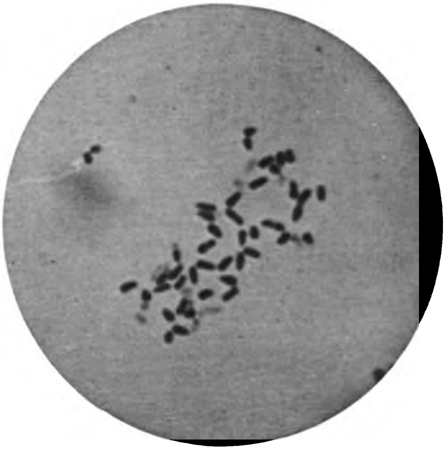

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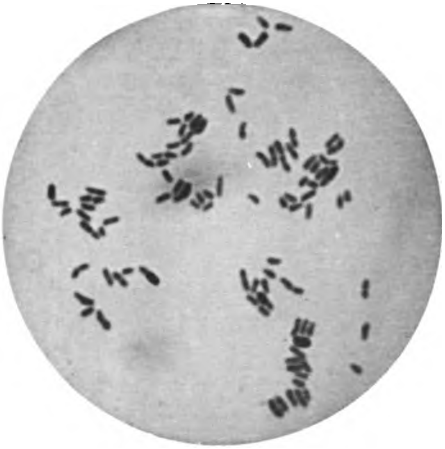

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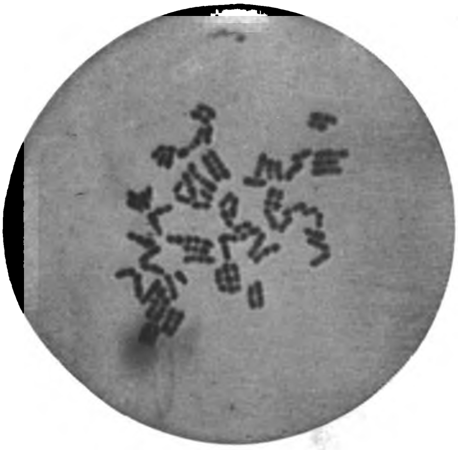

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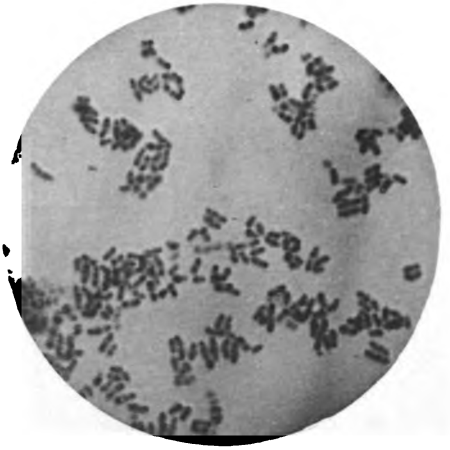

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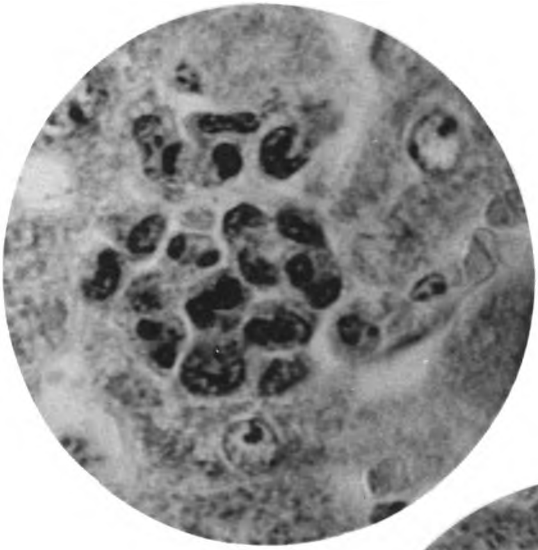
Denny. B. Diphtheriae, etc.



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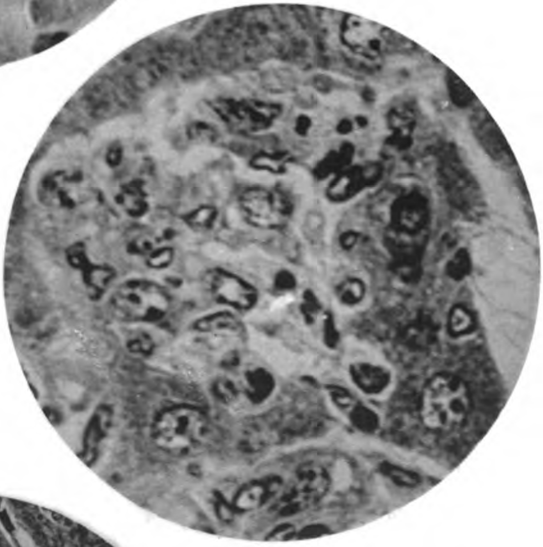

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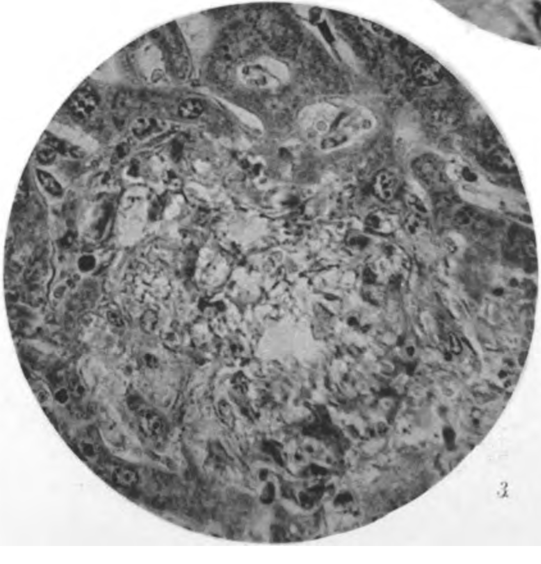
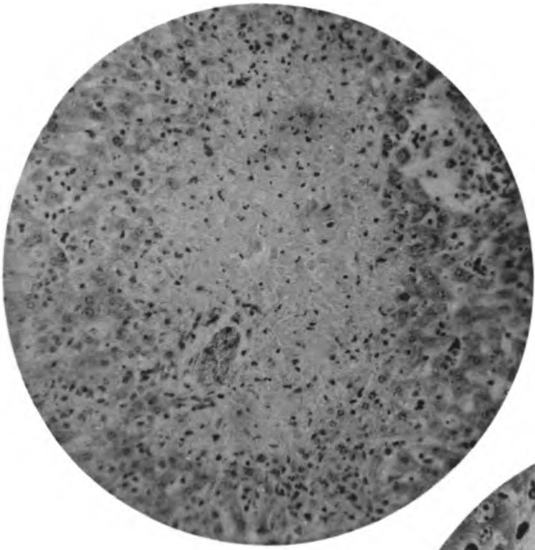


3.

Boxmeyer.

Liver Necrosis.



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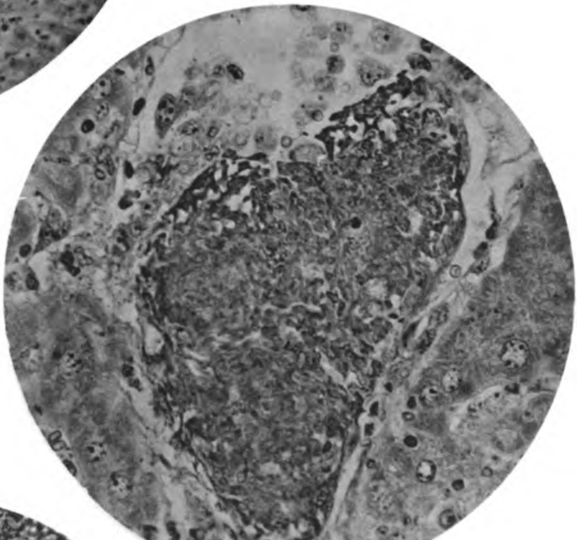

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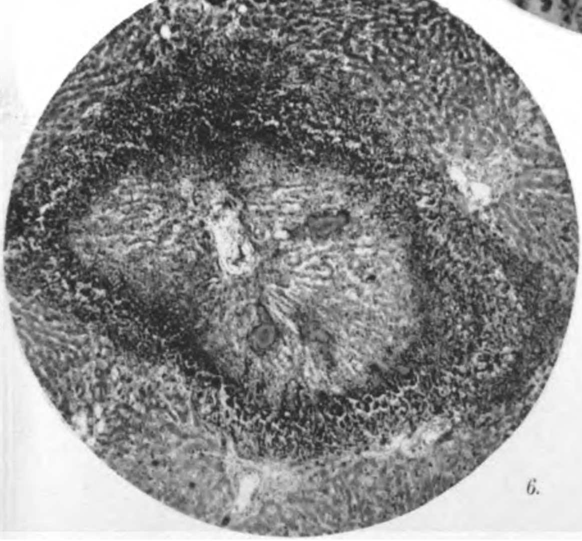


6.

Boxmeyer.

Liver Necrosis.

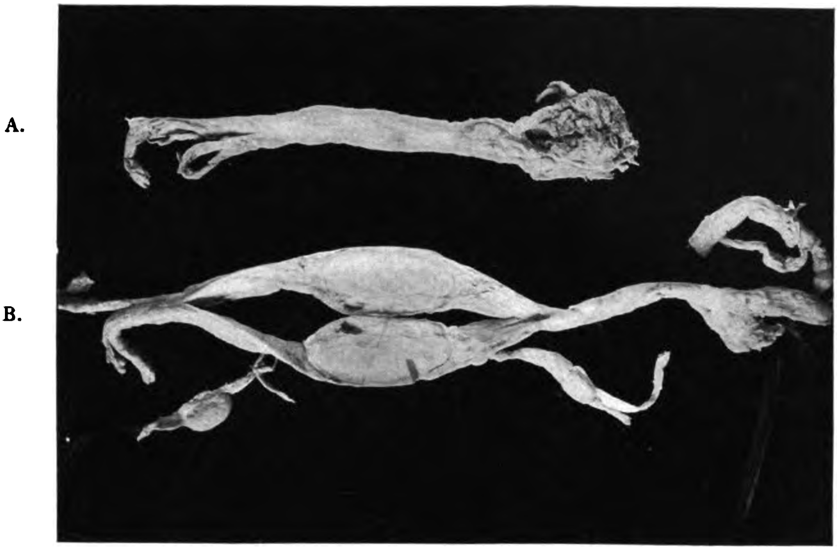


FIG. 1.
A.—Right sciatic nerve. B.—Left sciatic nerve.

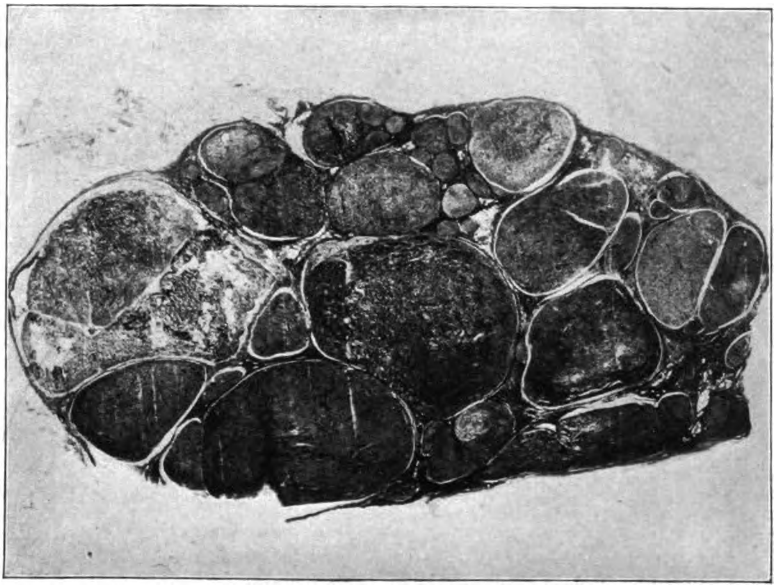


FIG. 2.
Cross-section of sciatic nerve in neuro-fibroma with sarcoma.

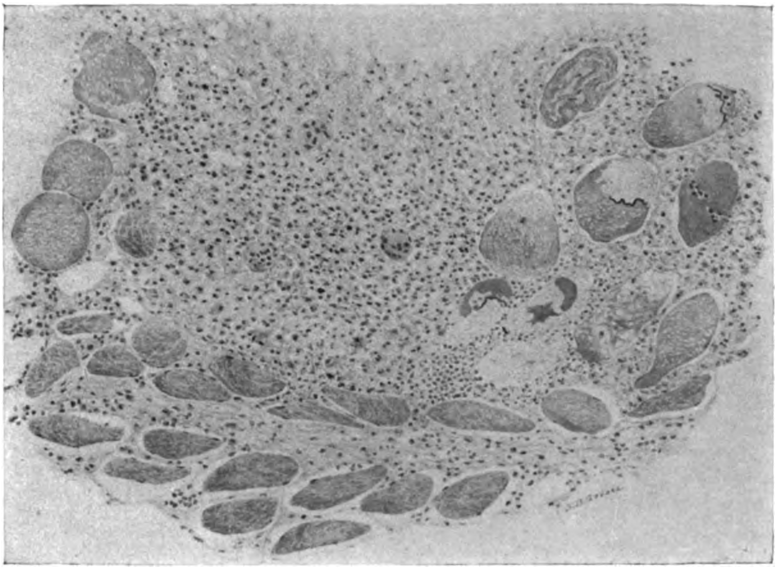


FIG. 1.

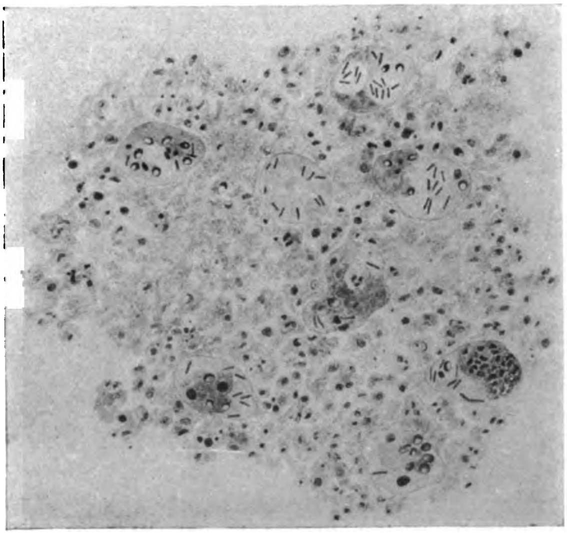
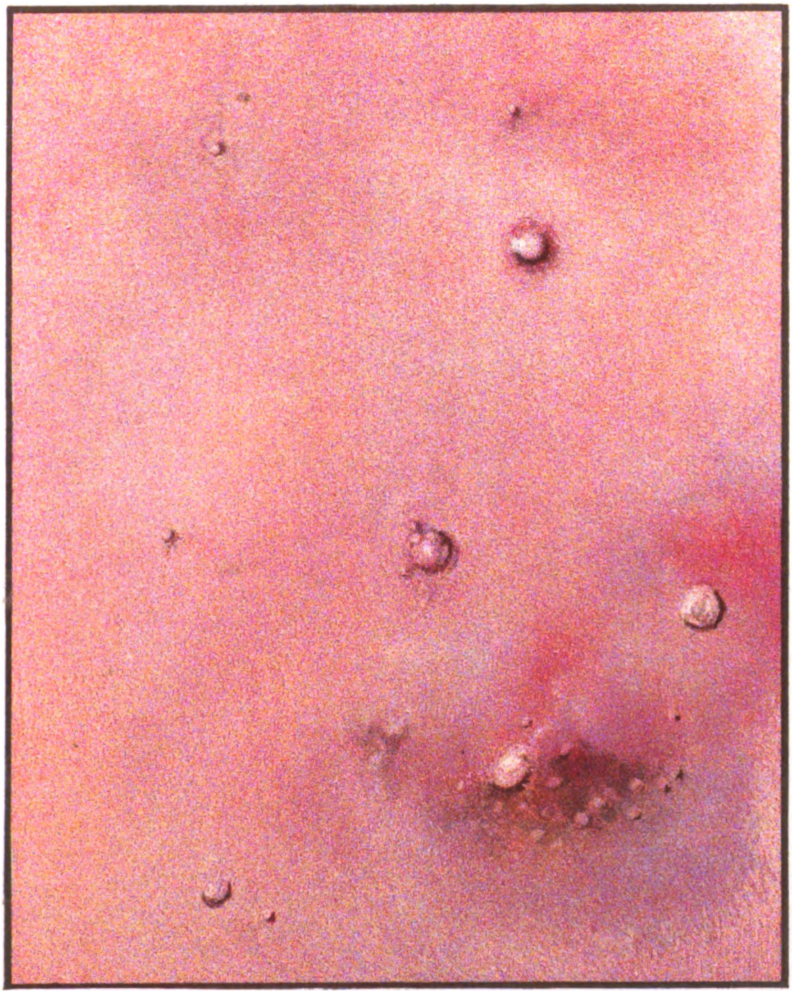


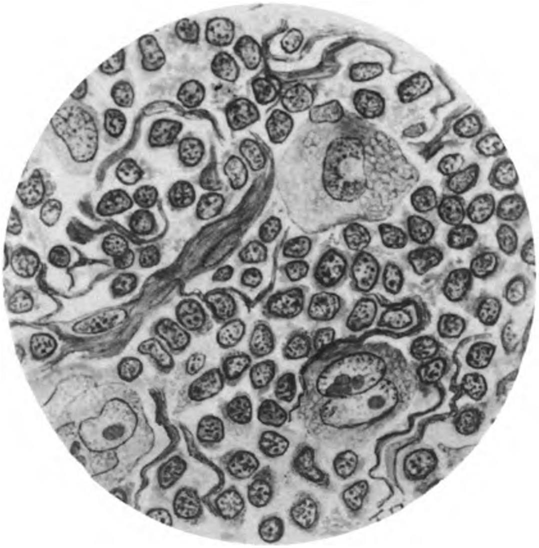
FIG. 2.

COLEMAN AND EWING. GLANDERS.



Coleman and Ewing. Cutaneous Lesion of Glanders.

J. OTTMANN LITH. CO.



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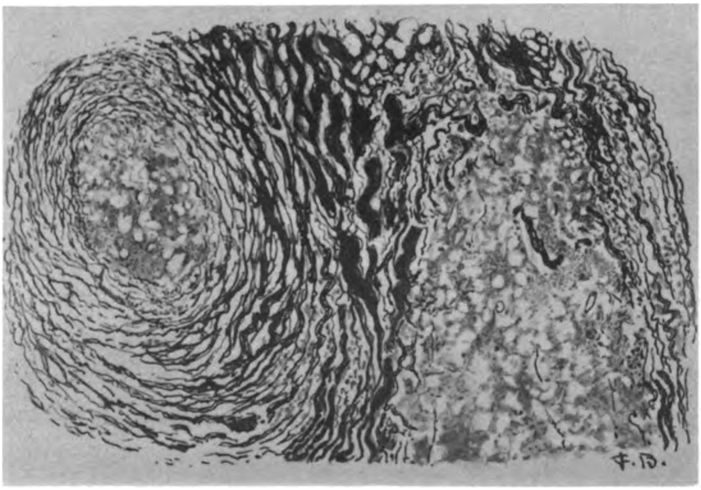
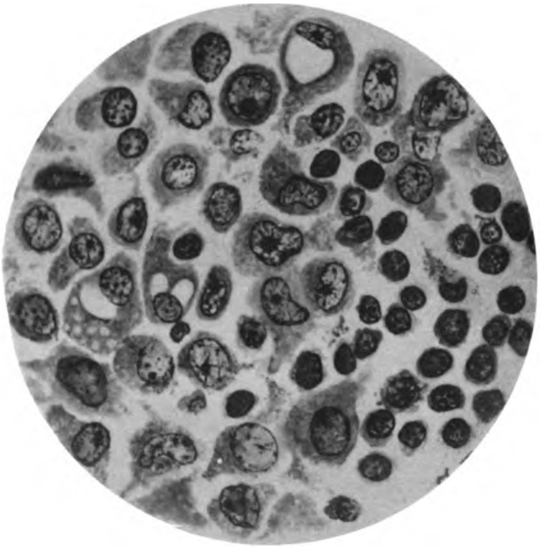


2

Simmons. Hodgkin's disease.



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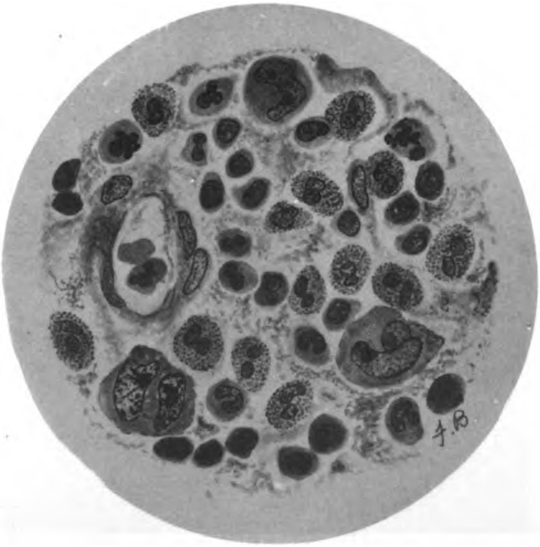

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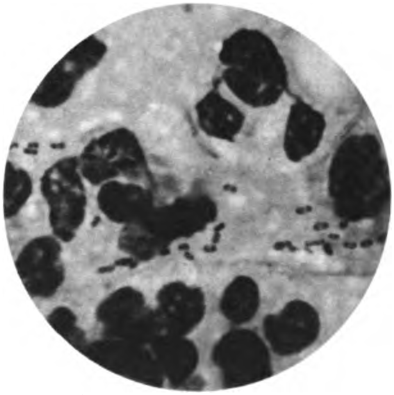
Simmons.

Hodgkin's disease.



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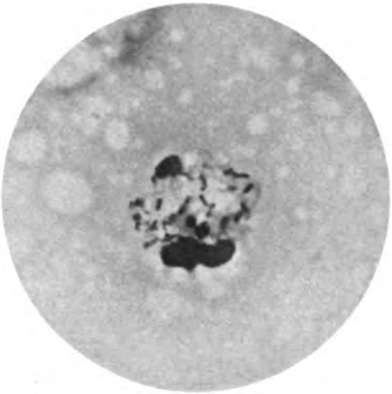

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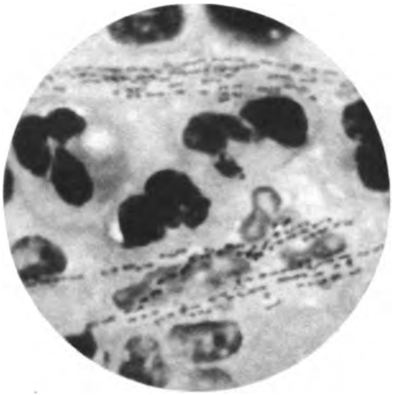

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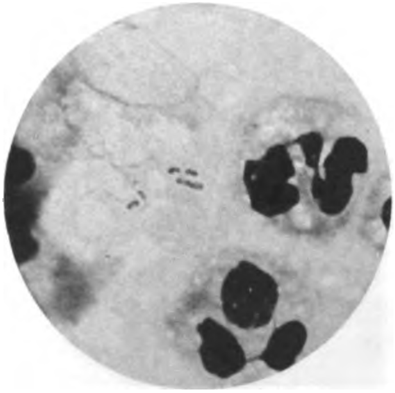

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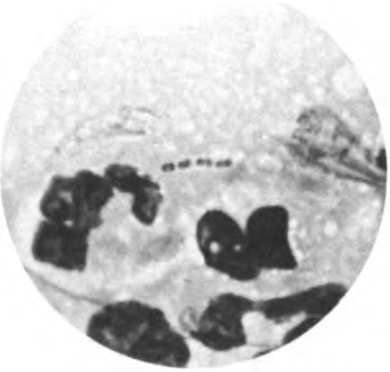
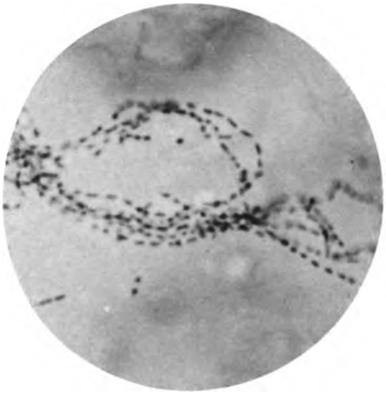


5

Davis.

Chancroid Bacillus.



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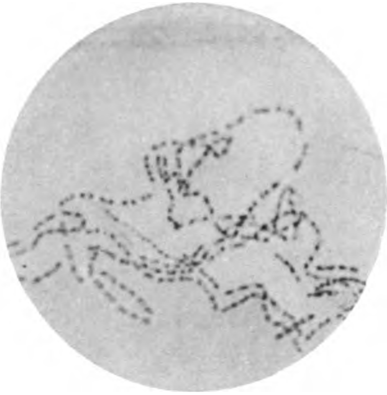

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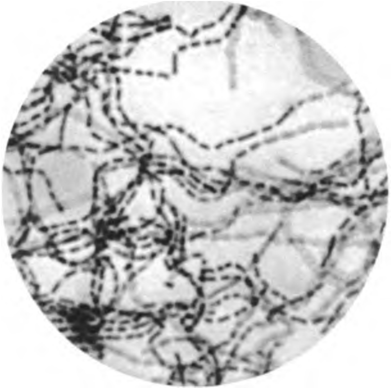

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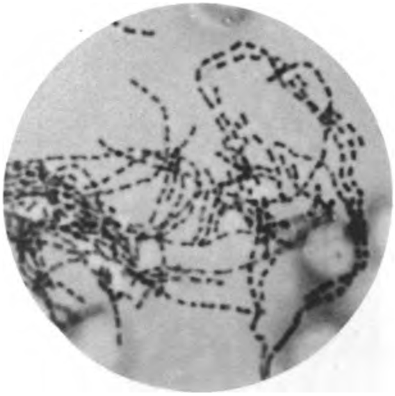

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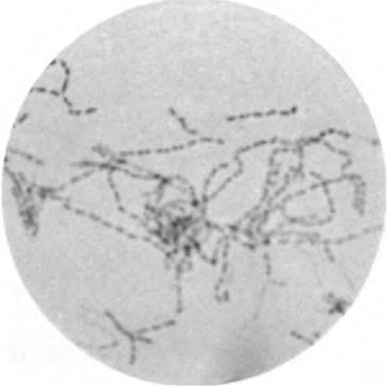

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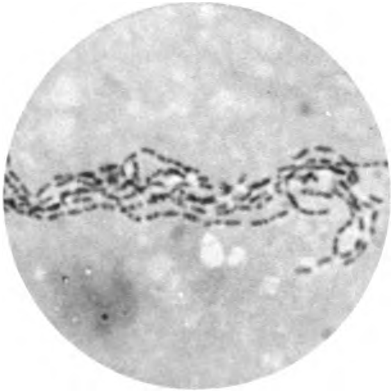
Chancroid bacillus.

Davis.



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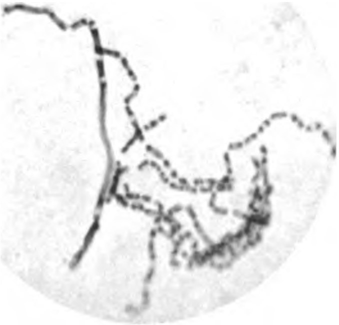

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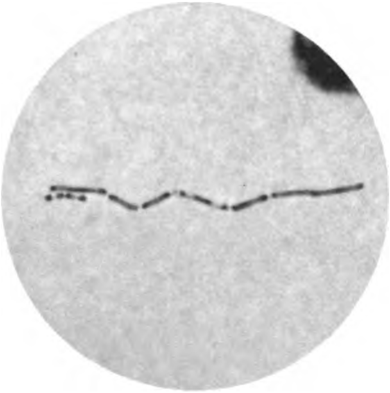

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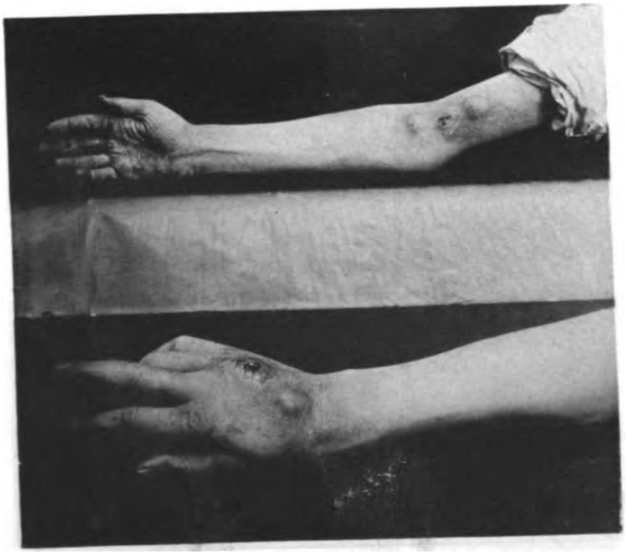
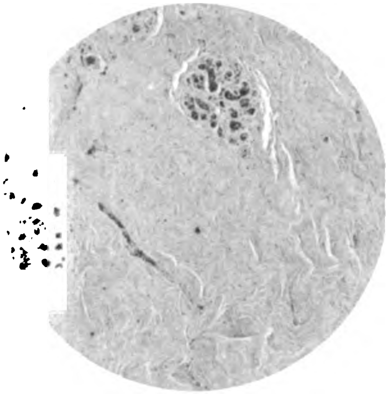


14

Davis.

Chancroid bacillus.

[illegible]



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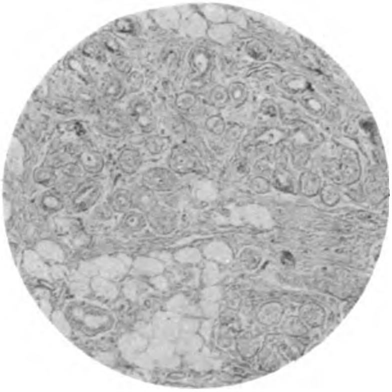

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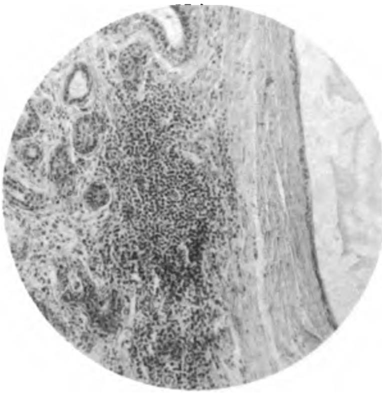

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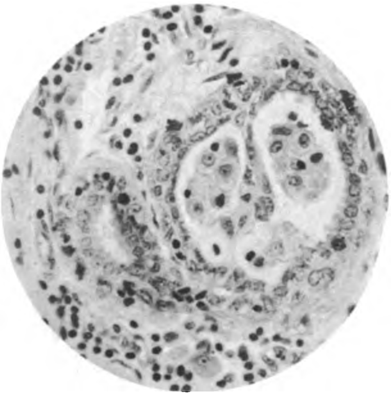

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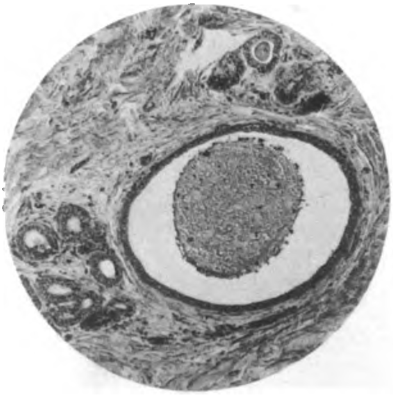

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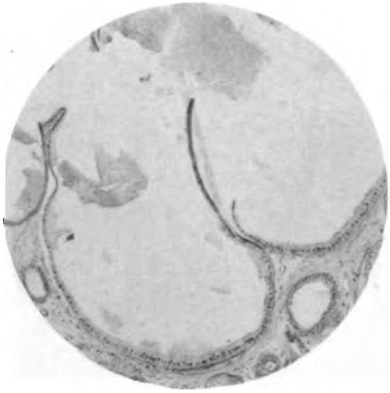

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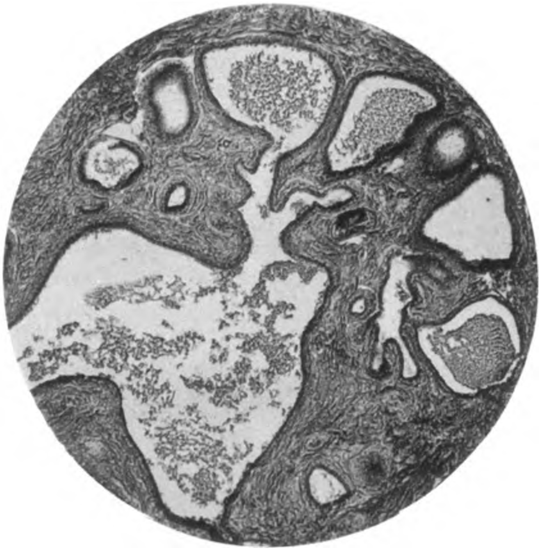

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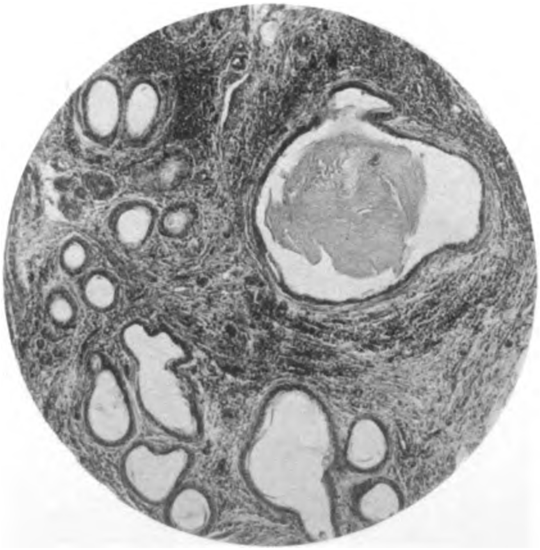
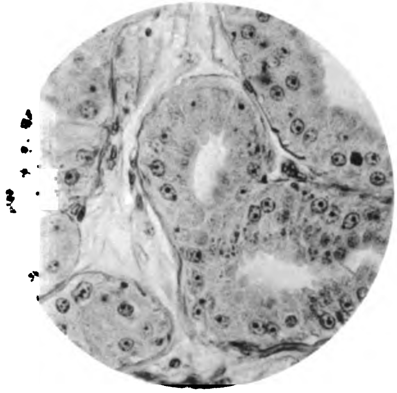


2

Greenough and Hartwell. Cystic mastitis



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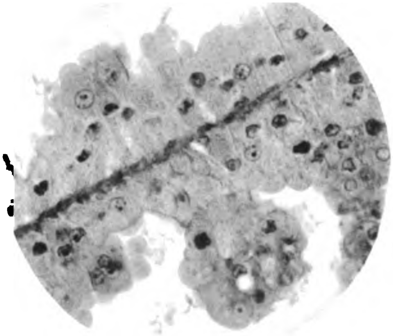

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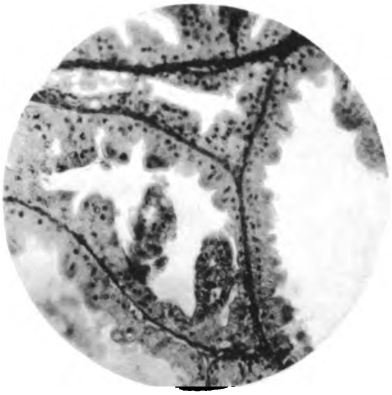

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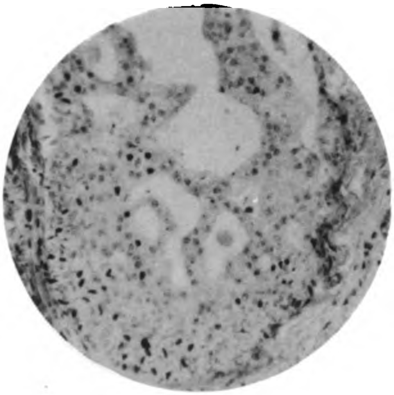

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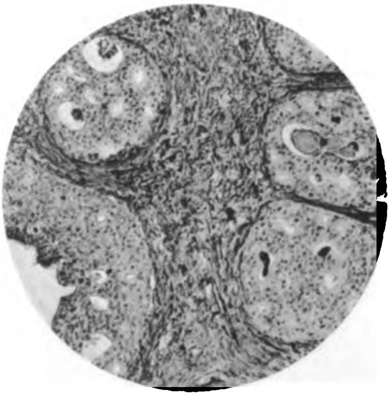

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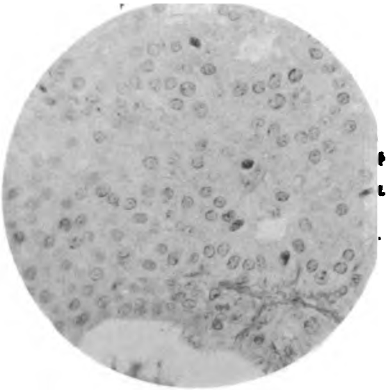
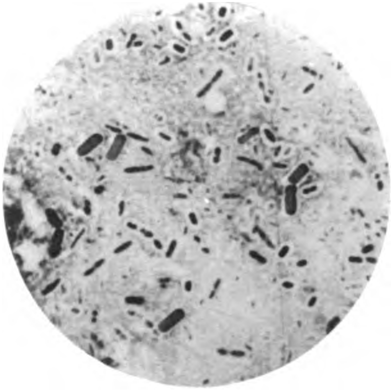


6

Greenough and Hartwell.

Cystic mastitis



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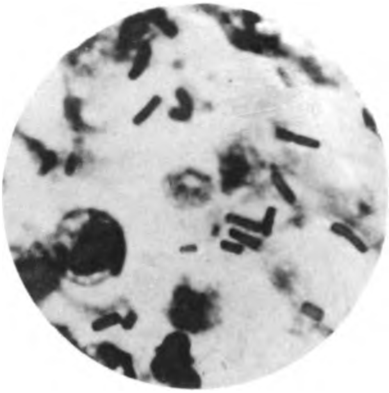

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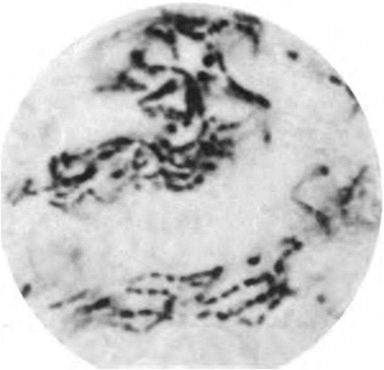

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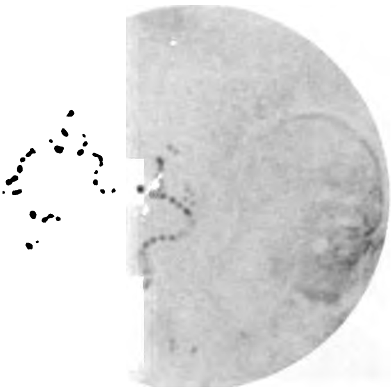

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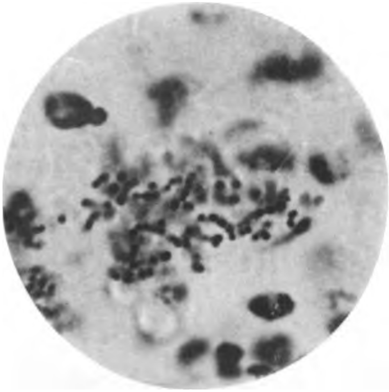

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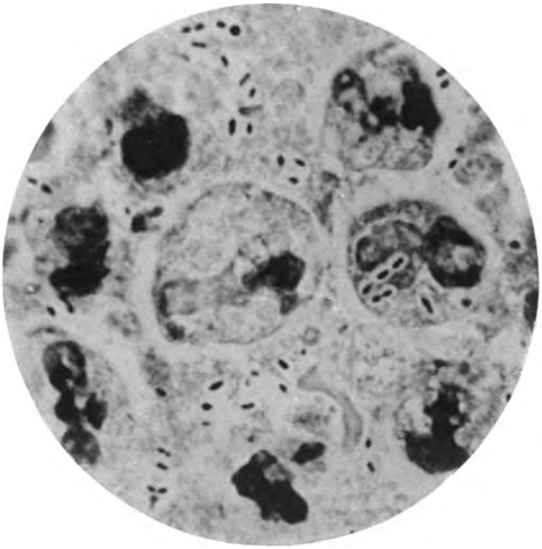
[illegible]

Peritoneal exudate.



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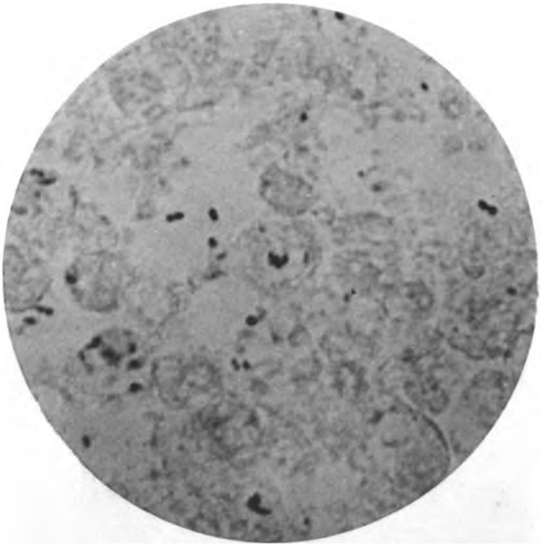


2

Manahan.

Peritoneal exudate.

[illegible]

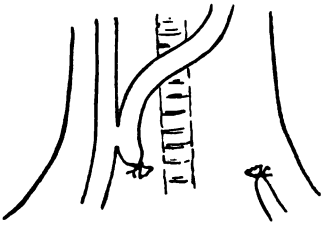


FIG. 1.

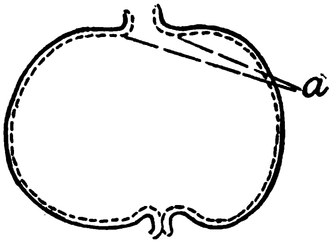


FIG. 2.

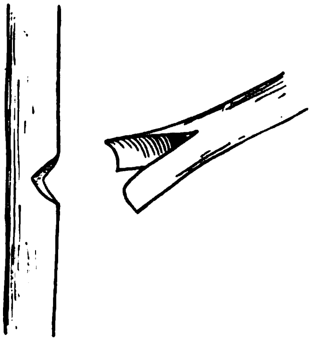


FIG. 3.

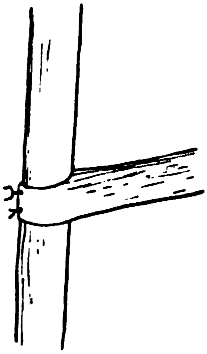


FIG. 4.

HUBBARD. TRANSPLANTATION OF ARTERIES.

FIG. 1.

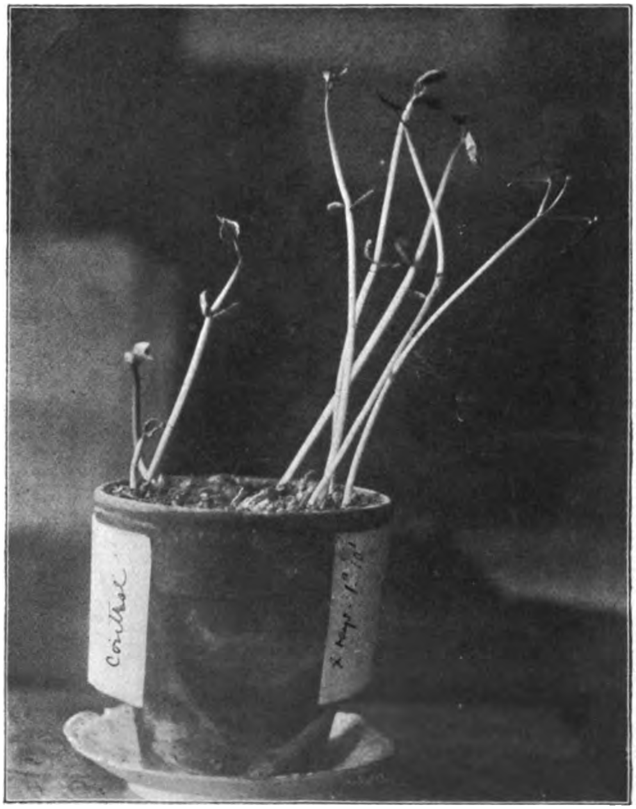


FIG. 2.

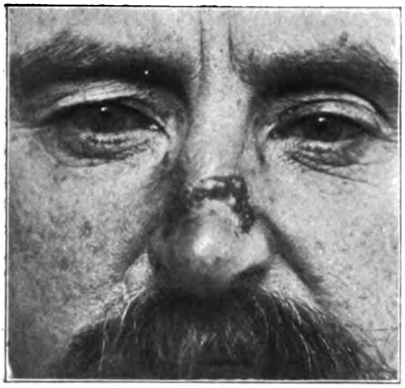


FIG. 1.

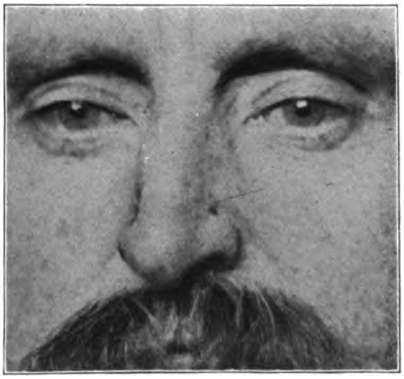


FIG. 2.

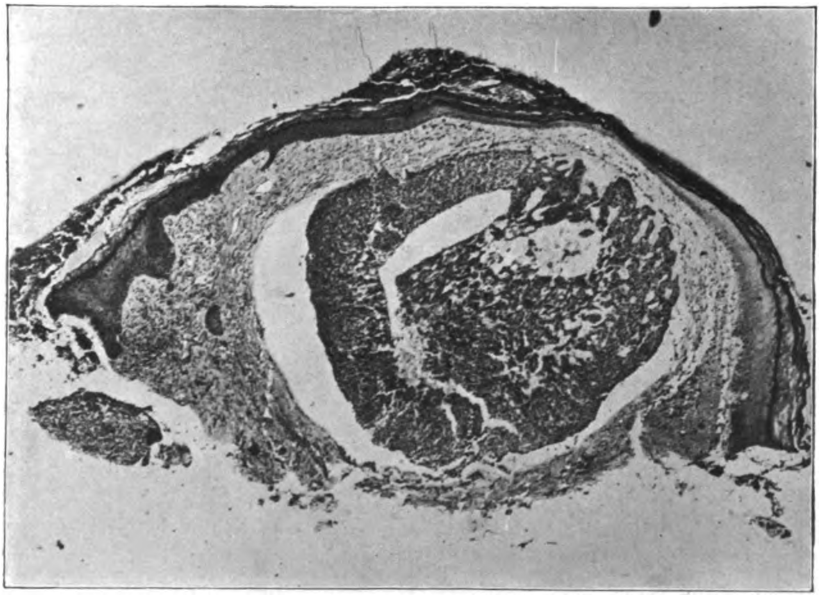
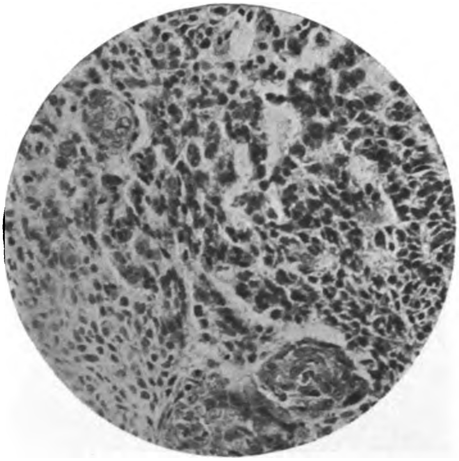


FIG. 3.



ALLEN. FIG. 4. X-RAYS AND LIVING TISSUE.

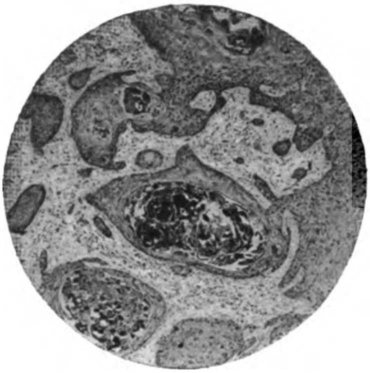


FIG. 1.

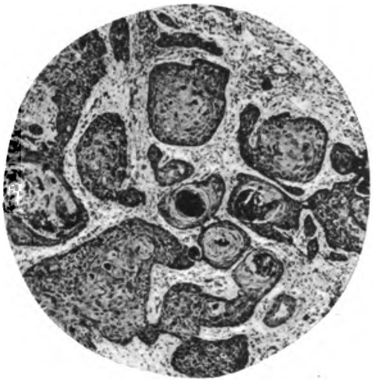


FIG. 2.

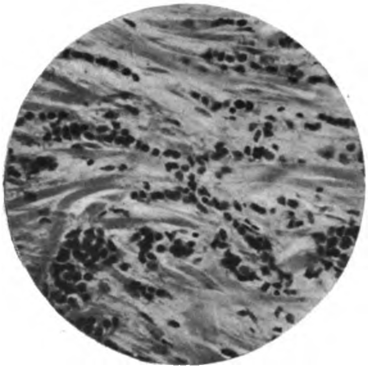


FIG. 3.

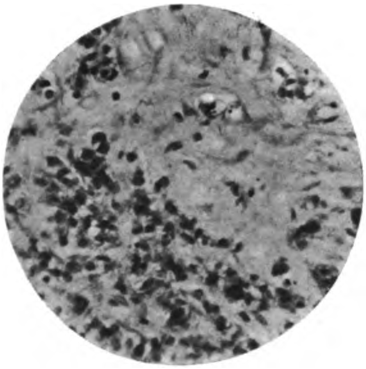


FIG. 4.

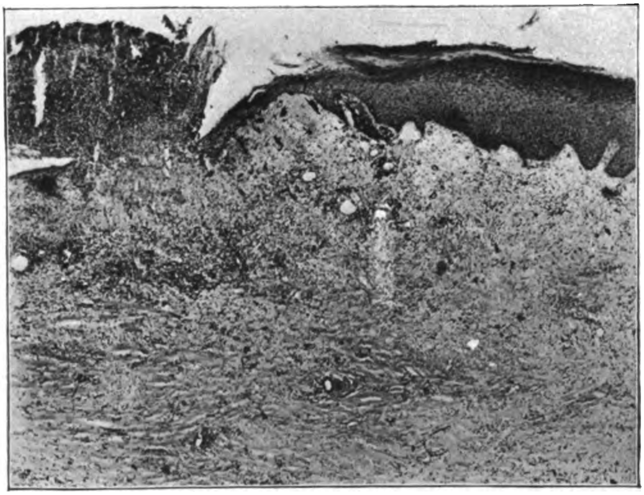


FIG. 5.

ALLEN. X-RAYS AND LIVING TISSUE.

3 2044 106 227 119